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PARASITOLOGY

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Volume IX 1916–17

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DAWSON'S OF PALL MALL

16, PALL MALL, LONDON, S.W. 1.

1959

OBSERVATIONS ON THE INFLUENCE OF SALT AND OTHER AGENTS IN MODIFYING THE LARVAL DEVELOPMENT OF THE HOOK-WORMS: *ANKYLOSTOMA DUODENALE* AND *NECATOR AMERICANUS.*

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INTRODUCTION.

IT is unnecessary to enlarge on the wide-spread distribution of ankylostomiasis or hook-worm anaemia. Occurring as it does not only throughout the Tropics but also in many countries of the temperate zones, it is a disease of the highest importance. The ravages which it has made amongst the population of these regions have given rise, especially during the last decade, to much concern and have led to the adoption of many active administrative and sanitary measures. By far the most considerable efforts in this direction have been made in the southern parts of the United States of America and in the West Indies where many workers are engaged exclusively in combating the spread of the disease. The magnitude of their task can be gathered from the fact that the disease has a firm hold upon a large proportion of the population both white and coloured. Efforts of a similar nature have been made throughout the British Empire and in other countries, but these have been neither so extensive nor so highly organised as those in America. The results of these efforts have been, on the whole, decidedly successful and encouraging and have shown that where the problem is strenuously and efficiently tackled the spread of the disease can be checked, its severity mitigated and the health of the population restored, even though it has been found impossible to absolutely eradicate the parasite throughout any large extent of country.

In all regions in which hook-worm disease is prevalent there exist large numbers of persons who are infected with the worms but who

show few or no signs of the disease. These individuals do not seek medical attention, receive no treatment and so continue to spread infection. This undoubtedly constitutes the chief difficulty in the whole problem. In the great majority of cases it is comparatively an easy matter to rid the patient of the disease, though exceptionally a patient remains insusceptible to all forms of treatment. Again there are many cases in which a cure can be effected, but where it is found impossible to remove the last few worms. Such cases may thus remain potential disseminators of infection for several years. There are many instances too, where no symptoms of disease have been exhibited and in which the ordinary routine examination has failed to detect the presence of worms. Such cases are a source of danger. This danger is accentuated by the fact that the worms may remain alive in the intestine for five or six years after the person removes to an uninfected area, and by the fact that fully developed larvae can survive for many months under favourable conditions, which are not at all difficult to find.

It would appear from the foregoing that, in a highly infected community, medicinal treatment, to reach its maximum efficiency, must be periodic and routine, and that all persons in the community should be subjected to inspection and those found infected should receive adequate treatment. Needless to say this is a counsel of perfection almost impossible to achieve except in the smallest of communities, but in any case in the attempt to effect complete eradication of the parasites, medicinal treatment is secondary in importance to general hygienic measures.

The excessive difficulty of attaining complete eradication is brought forcibly to notice by the Westphalian epidemic in Germany. In the coal mines there the parasite spread with considerable rapidity so that eventually almost all the mines became infected and a comparatively large proportion suffered considerably. Prompt measures very quickly effected an improvement so that in a few years the cases of infection and illness were greatly reduced in number. In spite, however, of the energetic action of a large staff of competent medical officers the introduction of the most stringent sanitary precautions and the expenditure of over £200,000, it was found impossible to remove the parasite entirely from the mines, and although eventually no cases of actual illness occurred, yet a certain small percentage (3 %) of the men remained lightly infected. There is little doubt that were the sanitary measures relaxed and the old condition of affairs reverted to, the mines would,

in the course of time, again become heavily infected. The above-mentioned experience occurred in a comparatively restricted community in which the infected individuals, being under disciplinary control, could be removed from the scene of infection, *i.e.* the mines, and debarred from returning until such time as the authorities considered them to be in a suitable condition. If the result, imperfect as it was, was difficult to attain under such circumstances, how much more formidable must be the task in an extensive community in which the only control is public opinion fostered, it may be, by well directed educational and preventive efforts, but supported only by a more or less imperfectly administered public health law!

It must be added, however, that the more restricted the area to which a certain number of infected persons are confined the more heavily is that area likely to be polluted and the more intense will the infection become.

In Queensland both species of hook-worm are widely distributed but they are confined chiefly, if not entirely, to the coastal belt extending throughout a length of over one thousand miles. In the drier regions inland the parasite appears to be entirely absent and the few cases of infection occurring there have probably been imported from infected areas. It must be noted, however, that so far as I am aware no systematic search has yet been made inland, and although practically no cases of disease have been recorded that fact does not preclude the possibility of infection being present. The indigenous population of North Queensland is comparatively small and as they do not come a great deal into contact with the white people, except in particular localities, their influence in spreading the disease cannot be very great. I am informed by Dr Breinl that during the past six years he has not observed a single case of the disease in an aboriginal.

There is, however, a considerable number of immigrant people, Chinese, Cingalese, etc. who find occupation as gardeners, cooks, laundrymen and so forth. These live in intimate contact with the rest of the population and coming, as many of them have done, from highly infected countries, there can be but little question that they have done much to introduce and spread the disease in North Queensland. Among the white population, again, it is noticeable that a certain proportion of the cases of infection occur among the families of immigrants from southern Europe, in many parts of which Ankylostomiasis is fairly common. It is quite possible that many of these people have imported along with them a number of hook-worms.

The incidence of infection in North Queensland does not differ from that in other infected countries. As usual children are more liable to infection, but adults are by no means exempt and indeed some of the most severe cases occur in the latter.

The people of North Queensland are not below the average in intelligence but they have that freedom and carelessness of temperament frequently met with amongst white people in tropical countries. On the other hand the absence of cheap coloured labour makes it necessary that almost everybody in the country should do a certain amount of manual labour. While this is good in so far as it ensures that nearly everyone shall have a fair amount of bodily exercise, yet it results, at the same time, in many of the more menial tasks being neglected or inefficiently performed. In nothing is this more noticeable than in the ordinary hygienic arrangements. The disposal of night soil and household refuse is usually very tardy. Tin cans and other discarded articles of household use are allowed to accumulate indiscriminately and for indefinite periods. Rainwater collecting in those forms frequent breeding places for domestic mosquitoes. Bath and sink washings are allowed to escape on to any convenient spot in the garden or yard, or they are carried away to some distance in an open drain. Although the danger from these sources may not be very great, still they constitute favourable places for the development of hook-worm larvae should such gain access, and as it is a common practice for children to go about barefooted the risk of infection is not negligible. A more important danger is met with during the rainy season when for days and even weeks the ground may remain thoroughly sodden and numerous pools of various sizes are formed. This, needless to say, provides the ideal condition for the development of the parasites. It is a matter of observation that the great majority of cases of heavy infection occur near the end of the rainy season and for two months or so thereafter.

The original source of infection is faecal matter charged with eggs from an already infected person. This, however, is not immediately infective, for the eggs and the larvae which are hatched from them must undergo a process of development which usually takes from five to seven days. Towards the end of this period the larvae begin to escape from the faecal matter provided the environment is favourable, *i.e.* containing sufficient moisture and without deleterious conditions being present. Thereafter they may remain alive possibly for as long as a year, or even longer, and may wander over very considerable areas.

How far they may spread in this manner is not definitely known, but laboratory experiments show that their power and rate of progression are by no means small. In the absence of moisture, however, the larvae are unable to leave the faecal matter and they perish as soon as it dries up. As a rule this does not take more than a few days or weeks, but if, during that period, moisture is supplied in the shape of rain or otherwise, many of the larvae are able to continue their development and eventually make their escape.

It is apparent from what has been said that if measures be taken to prevent the escape and spread of the larvae then there is no risk of infection except by actual handling or contact with the faecal matter. The simplest method of effecting this is to have the faeces deposited in an impervious receptacle which is kept dry. In the course of time the larvae will die off naturally, or they can be destroyed at a convenient time and place.

In North Queensland there is undoubtedly a well-informed public opinion on matters affecting the welfare of the community, but it has not yet assumed any very stable shape. This is only natural in so comparatively young a country. Opinion on matters affecting the public health has materialised sufficiently to be embodied in a system of laws and regulations which, theoretically, are probably not surpassed by those of any other country in the world. The provisions are most comprehensively and efficiently framed, but the machinery is lacking for their successful execution. This is not altogether to be wondered at. The conditions met with throughout the length and breadth of Queensland are so numerous and varied and the population in large parts is so sparse and scattered that it would seem impossible for the present to maintain a staff of officials sufficiently large to deal exhaustively with all the difficulties and problems that arise. The obstacles are particularly great in cases into which commercial considerations enter, and there is little doubt that a disproportionate part of the time and energy at the disposal of the Health Department has been spent in those matters in which the actual health of the community is not seriously endangered. So far as I can judge less attention has been devoted to diseases, particularly to those of an infectious or communicable nature. In the case of such diseases the commercial element occupies a comparatively subsidiary place. The prevention of hook-worm disease is a case in point. The provision of proper sanitary accommodation and of boots or shoes for the children at least during the wet season would not entail a heavy expenditure

upon the part of the householder, but to insure that these provisions are stringently carried out would involve a large amount of unremitting labour on the part of the sanitary officers. This labour could be reduced to a minimum by the intelligent co-operation of the general public. The insistence on the danger of defaecating elsewhere than in properly appointed places and of walking barefooted on wet ground or in pools of water would deter some persons from spreading infection and others from incurring it.

To sum up, the chief hope of eradicating and preventing hook-worm disease lies in thorough sanitary measures, rigorously administered.

The use of disinfectants.

It has been a not infrequent question, put by public health bodies and by others interested in the matter, whether disinfectants or other substances could be employed to check the spread of Ankylostomiasis. This question appears of some importance but it loses much of its weight in view of what has been said above as to the simple measures necessary for the purpose. In spite of that it must be admitted that it would be valuable to have at our command some secondary and auxiliary measures.

The use of ordinary disinfectants has most naturally been suggested, and a considerable amount of experimental work has been done on this matter. The results have shown that while fairly weak solutions of disinfectants such as izal or corrosive sublimate will kill the young larvae within a few hours, yet the eggs and fully grown larvae are much more resistant. These results, however, are based chiefly on laboratory experiments in which the disinfectant solutions have been kept in continuous and intimate contact with the infected material, and they are not altogether applicable to natural conditions. Under the latter conditions, *e.g.* in the case of a faecal deposit, the disinfectant would percolate through and drain away from the infected material in a very short time and so the effect would, to a large extent, be lost. Even in a closed receptacle only that part of the faeces in touch with the disinfectant would be immediately affected, although in the course of a week or a fortnight most of the larvae from above would find their way into the fluid and eventually be destroyed. For the purpose of preventing the spread of hook-worms it is unnecessary to add disinfectants to a properly constructed sanitary pail, though of course their use for other reasons is

highly desirable. To add disinfectants to a defective or leaking pail would be very useful but it would be very much more expensive than having the pail renewed or put in proper order. The application of disinfectants to isolated deposits of faecal material would not always ensure the destruction of the parasites, and it would be much less satisfactory than removing the deposits and having them destroyed or placed in a suitable receptacle. In the case of large areas of country, such as plantations where larvae may occur in great numbers and infection take place, it would be much more expensive and laborious to employ disinfectants than to enforce sanitary cleanliness and the use of proper footwear, though of course it would be helpful. There is the added objection that the continuous use of disinfectants would have a deleterious effect upon the soil and the growth of vegetation.

Influence of Salt.

Other substances which are not usually regarded as noxious have from time to time been suggested. Amongst these is common salt (sodium chloride). This suggestion is a result both of natural observation and of experimental work. It was observed for instance, in Cornwall, that while many of the mines were heavily infected, others escaped altogether. On investigation the most probable explanation was found to be that the uninfected mines were partly under the sea and that sea-water had percolated into them. As a result the water in these mines was found to be heavily charged with salt (up to 2 or 3 %) while in the other mines the amount of salt in the water was comparatively low. Similar observations have been made in mines in many other parts of Europe. Later experimental work appeared to justify this conclusion. This matter will be referred to in greater detail in the following pages.

The Life-History of the Hook-Worms under Normal Conditions.

While the literature on the subject of hook-worm disease and its many medical aspects is exceedingly extensive, that dealing with the minute details of the parasites and their life-history is much more circumscribed. A general account of these is to be found in all text-books of Parasitology and Tropical Medicine, but for the purpose of the present paper a much more detailed *résumé* is necessary. The most comprehensive work on the subject is that by Looss (1905 and

1911) who discusses nearly every aspect of the subject except the purely medical treatment. This work is unfortunately not usually accessible to the general reader, but a short abstract has appeared in the *British Medical Journal* (1912).

From the point of view of preventive medicine one of the most interesting and useful contributions to the subject is that of Boycott (1911) who summarises the results which he and Haldane obtained during and after the Cornwall outbreak (1902–1905). This paper also includes a useful comparative study of the results obtained by other observers.

Since then the most important experimental work on the subject is that by Fülleborn (1914).

It is unnecessary for the present to make mention of other papers dealing with the matter of life-history and development. These will be referred to as occasion arises.

As described in general works on Parasitology the normal life-history of the hook-worms is briefly as follows:

The adult worms in the human intestine produce eggs in considerable numbers, more or less constantly throughout their sexual life, but occasionally with intermission, the reason for which is not at present clearly understood. From clinical observation, however, it is well known that infected persons may show a large number of eggs in their faeces every day over a long period, but on one or more days there may be few or practically none at all. This is a frequent source of trouble in determining the existence of hook-worm infection, especially in cases which have received inadequate treatment.

The eggs which are produced by the adult worms are carried through the intestine and are voided along with the faecal matter in the ordinary course. These eggs are of a very small size, measuring only on an average $0{\cdot}06 \times 0{\cdot}04$ mm. They possess a very thin shell. Within this there is a thin yolk membrane which is quite transparent and inside this in the fresh state can be seen four approximately globular cells, the two larger being situated towards the poles. These cells are compressed against each other. Less commonly eggs with eight or more cells may be observed.

If, now, this faecal material be deposited in some suitable place the eggs will proceed to develop. Suitable conditions are not difficult to find in all tropical countries and in most sub-tropical countries. They are met with occasionally even in regions far removed from the tropics. The essential conditions are heat, moisture, air and food. The first

two, however, must be within certain limits. The most suitable temperature is from 65°–85° F. (18°–30° C.). Below this range of temperature, development is very slow; above it, development is very rapid but the eggs and larvae are liable to die.

With regard to the amount of moisture the conditions are somewhat more complicated. For the initial steps of development the most favourable condition is that in which the amount of moisture in the faecal material is maintained approximately constant, provided the air supply is not excluded. If the supply of air is deficient the development is retarded. An adequate food supply is necessary for the growth of the young larvae.

Under suitable conditions then the egg develops rapidly. The two cells originally present in the egg divide again and again and eventually form a small larva. When fully formed within the egg shell this larva measures about 0·25 mm. in length. By the end of 24 hours it proceeds to make its escape from the egg shell.

It commences to feed and continues to grow in size for the next four or five days. At the end of the second day, when about 0·4 mm. in length, it sheds its skin and assumes a slightly different shape. At the end of the fourth or fifth day the skin again begins to be detached but is not actually shed. It remains as a sort of flexible sheath enclosing the larva, but not interfering with its movements.

This constitutes the infective stage in the life-history of the worm, for it is only when they reach this stage that the larvae can infect another person. The larvae now measure about 0·6 mm. in length and are very actively motile. Unless they have been washed away previously from the faeces they now proceed to migrate to a more suitable locality, namely the nearest water pool or patch of moist ground. Provided even a trace of moisture be present they are capable of traversing considerable distances and may thus give rise to infection far from the place where the faeces were originally deposited.

How far and how rapidly they may wander in this fashion is not definitely known, but judging from laboratory experiences their rate is probably not less than five feet per hour, so that in the course of 24 hours they may have wandered 40 yards. This means that an area of 5000 square yards might become infected within 24 hours after the larvae started to migrate, *i.e.* within a week after the faeces were deposited.

These larvae when they reach this final stage have ceased to feed, and they can remain alive for months and even years under suitable

conditions, *i.e.* where there is a sufficiency of moisture and air and not too great heat. In the laboratory they have been kept alive for over 18 months in plain water at a temperature of about 60° F. It can hardly be doubted that they will live fully as long under natural conditions, unless it be that they are attacked and devoured by other animals such as aquatic insects. On this matter, however, we have no information.

After this stage no further progress in the development of the larvae can take place until the advent of a suitable host and a suitable opportunity for entry into that host. The most usual mode of entry seems undoubtedly to be by penetration of the skin, the commonest route is *via* the bare feet, but the hands and indeed any area of the body may be invaded. On the other hand infection may be acquired through the mouth by drinking water containing the larvae. At the present time it is generally considered that infection through the bare feet is by far the commonest method.

It is unnecessary here to enter into details of the controversy regarding skin infection. Suffice it to say that the discovery first made by Looss in 1898 has been confirmed by a large number of independent observers, and that the ranks of those who have opposed contradictory beliefs have been greatly thinned.

It is also unnecessary to deal here with the further remarkable life-history of the worm. All that need be said is that after penetrating the skin the larva bores its way into a lymphatic or blood vessel and is carried in the blood stream to the lungs. Here it penetrates into the air chambers and makes its way along the bronchial tubes into the trachea. Thence it passes over into the oesophagus and so down into the stomach and intestine where it completes its life cycle.

Life-History under Abnormal Conditions.

The matter of the foregoing section has had reference almost entirely to the life-history and development of the hook-worms under normal and natural conditions. A considerable number of disturbing factors may be introduced. As already mentioned the three chief factors concerned in development are air, temperature and moisture. The effects of these can, in most cases, be very readily estimated. There are, however, several other factors to be reckoned with, the effect of which cannot be so easily determined. The first of these is the condition of the eggs themselves on being passed.

It has been maintained by several observers that in some cases unfertilised eggs occur and that these will not develop. The occurrence of unfertilised eggs, however, is categorically denied by Looss. Such an explanation cannot, therefore, account for the non-development of the eggs in certain cases. While admitting the possibility that unfertilised eggs might occur very occasionally, Looss asserts that he has never once observed them nor, to his belief, has anyone else. Personally I have never met with any indications which would lead me to suspect the presence in fresh faeces of unfertilised eggs. Much more important appears to be the condition of the faecal material. It may vary in consistency from dry, hard and firm masses to the opposite extreme of an almost watery fluid. Normal, well-formed human faeces usually contain about 70–75 % of water. Where eggs are present in such faeces they can usually be readily cultivated. When less moisture is present in the faeces and consequently they are firmer and harder, cultures can still readily be made by the addition of sufficient water. It is generally in the case of soft diffluent faeces that difficulty is experienced in obtaining satisfactory cultures of larvae. These stools are usually of a light clayish consistency and are evil smelling. Frequently even in what appears to be a normal stool one is encountered which is conspicuous by its unnatural odour. In such cases there is difficulty in getting satisfactory cultures.

If such vile smelling or watery stools are allowed to remain as they are, they will in most cases, even though heavily charged with eggs, give a very poor culture of larvae or even none at all. This is an experience which has been encountered by most observers who have taken the trouble to investigate the matter, and it has been my experience, not only with human infection, but also with infection in dogs and cats. If, however, such faeces be carefully and repeatedly washed, and the eggs sedimented, it is frequently possible to obtain a rich culture of larvae from even the most unpromising of material. Looss has also found that when such faeces are thoroughly mixed with bone charcoal satisfactory cultures may not infrequently be obtained.

It may, with some confidence, be expected that such stools under natural circumstances will produce few, if any, larvae unless they are affected by outside influences such as rain.

It is not easy to advance any hypothesis with regard to the matter. It appears reasonable to assume that in such stools some substances are present, deleterious to the hook-worm eggs or larvae. Whether these be normal physiological substances produced under natural

conditions it is difficult to say. Abnormal stools are not a usual or even a common accompaniment of hook-worm infection. There is in fact no characteristic faecal condition in hook-worm disease, and the changes above noted must be regarded as having their origin in some intercurrent condition.

The subject is one that would require prolonged study and it is doubtful if the results would have much practical bearing on hook-worm disease.

Turning now to another possible abnormal condition, there is the nature and quality of the soil or land over which the larvae, after their escape from the faecal matter, are scattered. It has already been stated that fully developed larvae can exist for many months in ordinary tap water or rain water. They can also be kept alive for long periods in distilled water and in normal saline solution (0·75 %). Under natural circumstances the medium in which the larvae live is rain water with the addition of certain salts and other elements taken up from the ground. These will, in every case, depend on the nature of the ground. Only a limited number of observations have been made on this important matter, either under natural conditions or in the laboratory. It has been mentioned in an earlier part of this paper that common salt (sodium chloride) in the strength of about 2 or 3 % has been observed to have a deleterious effect upon the eggs and larvae of hook-worms. That this is not invariably the case will be shown later. This is practically the only salt on which observation or experiment has hitherto been made.

A few observations have been made on the effect of acid (sulphuric) and it has been shown that very weak solutions will kill both eggs and larvae.

There remains to be considered abnormal conditions of food supply, air supply, temperature and moisture.

These matters have been discussed very fully and lucidly by Looss and by Boycott and little more need be done here than to combine and summarise their results. Several other workers have made detached observations on these lines, but as their work has been carefully examined and criticised by Looss it is unnecessary to refer to them here. It may be added that I have personally tested most of the more important observations and have been able to confirm them except in cases which will be noted.

A proper and adequate supply of food is necessary for the growth of the larvae. Looss came to the conclusion that faeces derived from

a purely vegetable diet were unsuitable for the development of the larvae and that a certain small proportion of animal matter was essential. On the other hand faeces derived from a purely meat diet are unsuitable on account of the rapidity with which putrefaction takes place. Again, in very watery motions there may be an insufficiency of food, but this must be exceptional. In certain laboratory cultures, moreover, obtained by repeated washing and sedimentation it may so happen that an insufficient amount of suitable food is left for the larvae and in consequence their growth will be retarded and possibly altogether arrested.

This matter is of some importance in the experiments I am about to describe, as the cultures were usually obtained by the washing and sedimentation process. As, however, in every case controls were kept the results could be satisfactorily checked.

A certain small supply of air is necessary for the development of the eggs and the growth of the larvae. When the larvae, however, are fully grown and ensheathed they can withstand deprivation of air for considerable periods. The air supply may be cut off in several ways, as for instance in a hermetically sealed vessel or a tightly stoppered bottle. Again the faecal material may be buried under a thick layer of earth or covered with a layer of water. Even the bulk of the faecal material itself, if compact enough, may prevent the ingress of air to the interior of the mass and so arrest the development of the more deeply situated eggs.

The absence of air, however, though it arrests the development of the eggs, does not immediately kill them. They can survive for several days or even weeks, and if within that time they are supplied with air they may proceed to complete development. Looss has shown that in the absence of putrefaction and other deleterious conditions, the amount of air required for the complete development of the eggs and larvae may be quite small.

With regard to temperature it has already been stated that the most favourable is between 65° and 85° F., the optimum probably being about 75° F. Within these limits, other conditions being favourable, larvae generally reach their full development in 5–10 days. It is important, however, to know the absolute limits of temperature within which the eggs and larvae can survive and continue their development.

Boycott determined the higher limit as 100° F. but at temperatures over 90° F. he found that the majority of the eggs and larvae died. Looss, however, found that eggs and larvae would survive and develop

at a much higher temperature (113° F.) if not too prolonged and provided decomposition was prevented. Half an hour's exposure to a temperature of 130° F. can be withstood by the fully developed larvae without loss of vitality.

The lower limit of temperature was fixed by Boycott at 59° F. but Looss has shown that development can proceed, albeit slowly, at a temperature as low as 48° F., and that temperatures only a few degrees above freezing point do not kill the eggs provided the exposure is not too prolonged; development, however, is arrested during the time of exposure below 48° F. but proceeds again when the temperature rises above that point. The fully developed larvae are apparently much more resistant to cold for, according to Oliver (1910), they will withstand burial in snow for at least six days.

While having no reason to doubt the accuracy of Looss' observations I am inclined to regard them as of somewhat theoretical interest. From a practical point of view I must regard Boycott's opinion as representing the facts as they are presented to us naturally, *e.g.* in the matter of the geographical distribution of the worms. Otherwise it is difficult to understand why infection did not spread above-ground in the Cornish mining district during the summer months, when for six months the mean temperature is over 50° F.; nor again is it easy to understand why the infection has not spread into the southern parts of Australia, where even as far south as Melbourne the mean annual temperature is 58° F. It cannot be that other conditions apart from temperature are different, except it be some hitherto unrecognised condition, such, for instance, as the wet bulb temperature or the humidity of the atmosphere. It seems to me that while Looss' estimate of the critical temperature as 48° F. is probably correct *under the most favourable circumstances available*, yet the disadvantages of a less favourable natural environment necessitate a considerably higher temperature. Looss is slightly obsessed by the doctrine that what is natural in the biological world is best or most favourable, and he is inclined to lose sight of the fact that human ingenuity can sometimes improve on nature, as in the case of his charcoal method of rearing hook-worm larvae.

Little more than has already been done need be said on the subject of the necessity for moisture in the development of hook-worms. Only a very small quantity is necessary, while on the other hand a large excess is regarded as harmful. It is pointed out by Looss, however, that this is not due to the action of the water, as such, but is the result

of suffocation of the eggs or starvation of the growing larvae. Absolute desiccation is fatal to the eggs and larvae in all stages. The latter statement has led to some controversy, into which Looss enters very fully. My personal observations support his views on the subject.

One matter must be referred to in Looss' experiments. He makes mention of the fact that it is difficult to estimate the precise effect of any one particular agent or condition independently of other conditions. He lays special stress on the effect which decomposition or putrefaction may have in marring certain results and to this he attributes the apparent errors of certain other observers who used material in its natural state in their experiments. Looss himself conducted most of his experiments with faecal material mixed with animal charcoal which to a certain extent prevents or arrests putrefaction. While realising, however, and insisting upon the fact that the majority of laboratory experiments are more or less artificial and unnatural he does not sufficiently emphasise the fact that his charcoal method is perhaps one of the most unnatural of all, for it provides an environment for the developing eggs much more favourable than they would as a rule obtain in nature. Under natural conditions decomposition and putrefaction set in with greater or less rapidity. In this case the decomposition products make their escape to some extent in the shape of gases or are washed out by rain, whereas in a laboratory experiment these products are largely retained. It may, therefore, be concluded that while laboratory experiments with untreated faeces are less favourable than natural conditions, methods such as the admixture of charcoal are on the side of being more favourable.

Experimental Investigations.

The main object of the work detailed in this report has been to investigate and determine the action of common salt (sodium chloride) and other agents upon the development of hook-worm eggs and larvae in their various stages.

The practical point in the investigations was to determine if salt would be able to kill the eggs within a short time (24 hours) and so avoid the possibility of their being washed into more favourable environment by an intervening shower of rain or other cause.

The experimental work has been extended over a period of two years partly in the hope that as great a variety as possible of patients and of conditions might be obt ined; partly for the reason that the

number of patients available was not very great and of these only a small proportion were suitable for experimental purposes.

The material in nearly every case has come from hospital patients, and as the chief aim in such cases was curative, more than one sample of untreated faeces could rarely be obtained and in many cases even that could not be procured. No material was used from patients who were receiving treatment. A few samples of faeces were obtained from orphanage children and from school children, but these were, as a rule, unsatisfactory, being too small in quantity to permit of an extensive series of experiments and being moreover of uncertain age. In most of these cases the eggs had undergone a considerable amount of development.

During the course of these two years only thirty-three cases of hook-worm infection have been submitted to me for examination—but in fifteen of the cases the faeces were not obtained until after treatment had been started. Of the remainder only twelve were found suitable for experimentation. In five of these cases more than one series of experiments was undertaken.

These experiments being so few in number and the factors involved being so complicated, it follows that the results, even in cases where they appear definite, cannot altogether be regarded as of general application. The nature and composition of the faeces employed, their age, the variations in the temperature during the experiments, are all factors which could not be accurately determined.

According to Looss (p. 371) the conditions which generally prevail in laboratory cultures are much less favourable to the larvae than natural conditions. It must be remarked, however, that this is not necessarily the case, for in a covered laboratory culture the supply of moisture is kept relatively constant and falls off only very slowly: the temperature is also more uniform; while the supply of air in suitably selected vessels, except when kept in a closed incubator, is quite sufficient for the full development of the eggs and larvae. If the cultures are properly manipulated there is every reason to expect that a considerable proportion of the larvae will survive for at least a year.

Technique of Experiments.

The faeces, having been obtained as fresh as possible and examined for the presence of hook-worm eggs, were divided into separate portions, usually 2–4 grams in weight. Several small glass vessels were previously cleansed and accurately weighed. Into each of these a portion of

faeces was put and the vessels again weighed. The weight of faeces used in each experiment was thus known. In the earlier experiments the vessels used were small stout bottles with wide necks and loosely fitting corks, with wooden tops. In the later experiments, however, small glass pots with heavy glass tops were used. The entrance of air was ensured by introducing a small paper plug between the pot and the cover.

The various liquids and solids used were added in measured quantities so that an approximate idea could be obtained of the amount of material required. These substances were applied in the manner specified in the detailed account which follows.

At intervals of 24 hours small portions of the mixtures were removed and examined to determine the condition of the eggs. Fragments were also thoroughly and repeatedly washed with water and the residue spread out in small Petri dishes. These were examined at regular intervals, usually 24 hours, to ascertain the state of development of the larvae.

In most cases the experiments were continued until all the eggs were apparently dead and no larvae could be reared from them.

At this point it may be well to discuss the various methods of rearing hook-worm larvae.

The natural and, perhaps, ideal conditions for the development of hook-worm larvae have already been mentioned in the brief outline of the larval life-history, but it is not often easy or even desirable to reproduce these conditions in the laboratory. The nearest approach to this may be obtained by exposing the faeces in an open Petri dish and drenching them with water every 24 hours. With each drenching a certain number of eggs and larvae are washed out and these can be reared in separate dishes.

In practice this does not prove a particularly efficient method of rearing larvae. One objection to it is that the faeces dry up within 24 hours. That can be remedied by drenching every 12 hours or 8 hours. Another objection is that flies, especially house-flies, deposit their eggs on the faeces and the resulting larvae rapidly eat up the material. At the same time it must be noted that these fly larvae in their wanderings distribute the faeces over a wide area and in a thin layer, a circumstance which is particularly favourable for the development of hook-worm larvae. To what extent this may aid the dissemination of hook-worms under natural conditions remains to be investigated. In the present series of observations, however, the majority of experiments to which

fly larvae gained access were discarded. A few in which the number of fly larvae were small were allowed to pass after the larvae had been removed.

One of the simplest and most convenient methods of rearing hook-worm larvae is to place small portions of faeces in Petri dishes along with a small quantity of water. Every 24 hours the water is poured off and replaced by a fresh quantity. The water removed is thoroughly mixed with tap water, sedimented and the supernatant layer decanted or pipetted off. The sediment containing the eggs and larvae is cultivated and usually produces a good yield of larvae. In many cases, however, this method fails or gives poor results on account of the nature of the faeces, as already indicated.

A modification of the method above-mentioned is to place the portions of faeces on a small pad of filter paper or blotting paper and surround it with water. This usually keeps the water somewhat cleaner.

The method advocated by Looss is to mix the faecal material thoroughly with bone charcoal and spread the mixture evenly in Petri dishes. A firmly consistent mass is obtained which tends to become dry on the surface. When water is poured over this, at the end of three or four days a fairly copious supply of larvae can be washed off. Looss insists on the necessity of using bone charcoal. Wood charcoal is quite unsatisfactory. I have employed both materials in the cultivation of human and canine ankylostomes. With wood charcoal the results have invariably been unsatisfactory, and indeed in the majority of cases entirely negative. Even with bone charcoal the results were extremely disappointing, and this method of culture was abandoned in favour of others which gave more immediate and satisfactory results. As Looss has pointed out it is not always easy to obtain a suitable variety of charcoal.

A more satisfactory result was obtained by employing dry sterilised sand with which the faecal material was mixed in equal proportions.

One of the most useful methods is to spread a thin layer of faeces over the bottom of a Petri dish. In the cover of the dish a piece of moistened blotting or filter paper is placed. At the end of four or five days the migrating larvae, attracted by the moisture, make their way up into the cover of the dish and can be collected in a very clean condition.

Provided the upper half of the Petri dish can be made grease free and the correct amount of moisture obtained, the blotting paper is not essential.

By far the most expeditious method, however, of cultivating hook-worm larvae is the dilution and washing method.

In the course of the present experiments this was found of great value and, in fact, almost necessary.

The technique is as follows: a small portion of faeces is thoroughly mixed with a small quantity of water in a dish, and the dish then filled up with water. After an interval of five or ten minutes the water is carefully decanted or pipetted off and the process repeated. Depending on the nature of the faeces from three to five washings are usually necessary. After the final washing a thin layer of water containing eggs and debris is left at the bottom of the dish and this is incubated at a suitable temperature. In the majority of cases numerous larvae hatch out within 24 hours.

The above method is also useful for large amounts of faeces and although it is somewhat tedious it usually gives good results.

DETAILS OF EXPERIMENTS.

I. The first series of experiments was conducted with the view of determining the effect of salt solutions of various strengths on the eggs and larvae of hook-worms. Strengths of 2, 4 and 6 % were used and the solution was added in the proportion of 2 c.c. to each gramme of faeces. As controls two portions of faeces were treated with rain water, in one in the proportion of 2 c.c. to 1 gramme, and in the other in the proportion of 15 to 1. In addition a portion of untreated faeces was cultivated. In the experiments in which rain water was used the smaller proportion of water did not cover the faeces, while the larger proportion covered them to a depth of about a quarter of an inch. In the case of the salt solutions the faeces were merely surrounded, not covered. Within 24 hours films and cultures were made from each bottle of the series. In every case the ova were found to be living and from each of the cultures living larvae hatched out. A day later the process was repeated and again living ova and larvae were obtained. The following two days witnessed the same result. On the fifth day all the cultures were positive except that from the 6 % salt solution. On the seventh day the only negative culture was that on which the smaller quantity of rain water had been used. On the eighth day the cultures from the two stronger salt solutions were both negative and no further larvae could be cultivated from them. The other cultures were still positive. The faeces to which a small quantity of water had

been added were negative after this date, but the untreated faeces and those to which 2 % salt solution had been added were positive for a further two days. The faeces treated with the large bulk of water remained positive up to the 21st day when the experiment was stopped. The control sample was completely dried up on the 10th day.

The results will be readily seen in the following table:

		April, 1915									May, 1915			
	Date	22	23	24	25	26	27	28	29	30	1	2	4	12
	No. of Days	1	2	3	4	5	6	7	8	9	10	11	13	21
1.	Untreated faeces	+	+	+	+	+	+	+	+	+	+	−	−	−
2.	Faeces surrounded by rain water 2 c.c. to each gramme	+	+	+	+	+	+	−	+	−	−	−	−	−
3.	Faeces completely immersed in rain water 15 c.c. to each gramme	+	+	+	+	+	+	+	+	+	+	+	+	+
4.	Faeces surrounded by 6 % salt solution	+	+	+	+	+	−	+	−	−	−	−	−	−
5.	Faeces surrounded by 4 % salt solution	+	+	+	+	+	+	+	−	−	−	−	−	−
6.	Faeces surrounded by 2 % salt solution	+	+	+	+	+	+	+	+	−	+	−	−	−

The conclusions to be drawn from these experiments are that, under the conditions described, solutions of salt (sodium chloride) even as strong as 6 % cannot be relied on to destroy hook-worm eggs in faeces in less than a week and that such solutions are not more effective than rain water under the same conditions. It must be noted, however, that in general the development both of the eggs and the larvae was greatly retarded by the presence of the salt solution, so that few of the eggs hatched in less than two days and most of the larvae did not reach the ensheathed stage till after ten days had elapsed. It must also be remarked that the mortality amongst the eggs and larvae was much greater than under normal circumstances and that in most cases only a few larvae, sometimes only one or two, were hatched, and a large proportion of these died within a few hours.

In the case in which the faeces were entirely submerged in rain water the eggs were also very slow to develop and it was generally two to three days before the larvae hatched out and thereafter their growth was slow, many dying shortly after being hatched.

In the control untreated faeces the eggs developed with considerable

rapidity and after the first day numerous larvae could usually be obtained within 24 hours. These followed a normal course and were usually ensheathed within eight or nine days from the start of the experiment.

It should be mentioned that fly larvae obtained entrance into the plate cultures of Nos. 2 and 3 (23. iv. 15), but were not again met with on the plates. Bottles Nos. 2, 5 and 6, however, became infected with fly larvae but these were immediately removed. Nos. 1, 3 and 4 escaped contamination. This matter of fly contamination was a difficulty throughout the course of these experiments, but in the later series it was of much less frequent occurrence. As far as could be judged, however, it did not appear to affect the results.

While on this subject it may be well to refer to the question which has not infrequently been discussed, as to the part played by flies in the spread of parasitic worms.

This is a matter which I had the opportunity of investigating some years ago (Nicoll, 1911) and the conclusion arrived at was that while house-flies may play an important part in the spread of certain worm parasites, the limit of their power was determined by the size of the parasitic eggs. In the case of hook-worm eggs it would be a matter of extreme difficulty for a fly to swallow them. A few, however, might adhere to the legs and body and so be carried for a short distance. From all we know of the habits of flies it is almost certain that they would rid themselves of such a comparatively large encumbrance as a hook-worm egg before proceeding very far.

Again it has been suggested that the fly maggots might spread the eggs of worm parasites by passing them on to the adult fly. This suggestion was based on an unpublished observation of Stiles in the case of *Ascaris lumbricoides*. My personal experience has entirely failed to support the correctness of this observation. It must be admitted, however, that the matter has not been thoroughly investigated in the case of hook-worms. In none of the maggots which I have examined has any trace of hook-worm eggs or larvae been found. At the same time it must be remembered that house-flies can and do serve as natural intermediate hosts of worm parasites, for Ransom (1913) has shown that *Habronema muscae*, a nematode worm which infects the horse; passes its early stages in the larva, pupa and adult of the house-fly. How the young worms find entrance into the fly maggots, whether by being swallowed or by boring their way in, is not made clear by Ransom, who has evidently not been able to elucidate

this difficult point. He remarks on the difficulty himself and mentions the fact that several free-living and larval nematodes may frequently be found on the surface and in the intestine of fly maggots. If these larvae can be swallowed by the fly maggots there appears no reason why the same should not occur with hook-worm larvae. The point to be considered, however, is the ultimate fate of these larvae. It must be remembered that *Habronema* and *Ankylostoma* belong to widely different groups of nematodes, the former of which usually requires an intermediate host, while the latter has none. It is, therefore, impossible to draw any analogy between the behaviour of *Habronema* larvae in the fly and the larvae of *Ankylostoma*, for if Ransom's view be correct, the fly is the natural habitat of the former.

II. In the second series of experiments the effect of common salt in the solid state was investigated. The salt was simply sprinkled on the top of the faecal material. An average sample of the faeces was found to contain 71 % by weight of moisture. The quantities of faeces and salt used in each experiment were weighed. It was found most convenient to employ 3–5 grammes of faeces while the quantity of salt used varied from 0·4 to 9·1 grammes. The proportion of salt to faeces varied from 1 : 10 to 30 : 1 and the proportion of salt to solid matter in the faeces varied from 3 : 10 to 10 : 1.

As 1 c.c. of water will only dissolve about 0·36 gramme of salt and therefore 1 gramme of faeces can only absorb about 0·25 gramme of salt it is obvious that all the salt used in the experiments at the extreme end of the series could not possibly be absorbed by the faecal moisture. Thus in one experiment eleven times as much salt was used as could be absorbed, and therefore the bulk of the salt only acted as a covering. Even with the enormous quantities of salt, however, occasional living eggs could be found in the faeces after 24 hours and larvae could be hatched from them although their growth was greatly retarded and they usually died very quickly. Apparently the salt was absorbed extremely slowly and did not thoroughly permeate the faecal mass till more than 24 hours had elapsed.

With another sample of faeces, however, containing slightly more moisture (72·3 %), 1 gramme of salt sprinkled on 2 grammes of faeces was found to arrest the development of the eggs completely within 24 hours. When half the quantity of salt was added the eggs survived for one day but not for two days, while when the quantity was reduced to one-quarter (1 gramme to 8 grammes) the eggs lived for two days

but not for three. The eggs in the control untreated sample lived for at least a fortnight.

With the same sample of faeces a series of experiments was made with some highly hygroscopic salts such as potassium acetate, calcium chloride and silver nitrate, the object being to ascertain if the absorption of such highly soluble salts would prevent the development of the eggs. Calcium chloride and silver nitrate effectually did so when used in the proportion of 1 to 5, but potassium acetate which is an equally soluble salt failed to kill the eggs in 24 hours even when used in the proportion of 1 to 2. With this strength, however, the eggs did not survive for two days. From this it may be suspected that the greater effect of calcium chloride and silver nitrate was largely a poisonous one.

These three salts, it must be remarked, were used merely to test the effect of such salts and not with any view to their practical or general employment. It is obvious that they are quite unsuitable on a practical scale.

III. In this series of experiments weighed quantities of salt and faeces were used as before but the salt, instead of merely being sprinkled on the surface, was thoroughly mixed up with the faeces. Quantities of salt varying from 0·3 to 1·5 times the weight of the faeces were used. At intervals of 24 hours small portions of these mixtures were removed, thoroughly washed in water to free them from salt, and the residue plated. A control sample of faeces was also treated in the same way.

At the end of 24 hours examination of the faeces showed that most of the eggs in the control sample were alive and rapidly developing, while those in the other samples appeared to be all dead. Plate cultures from the control gave numerous active larvae while in the case of the others the results were negative, although the cultures were incubated for a week. At the end of another 24 hours cultures were again made. The results were the same as before with the important exception that the cultures from No. 1 (that with the least salt) produced a single vigorous larva which developed normally and eventually became ensheathed. At three further intervals of 24 hours cultures were again made, but except in the case of the control sample, no larvae were produced. The control sample remained productive for a considerable time afterwards.

The conclusions to be drawn from this series are that when faecal material is *thoroughly* mixed with half its weight of salt development

of hook-worm eggs is completely arrested within 24 hours and the eggs do not hatch. When the quantity of salt, however, is reduced to one-quarter or a third of the weight of the faeces a few eggs may survive its influence for two days and give rise to larvae. This amount of salt is sufficient to kill all the eggs within three days.

IV. This series of experiments was similar to the last but with material from another patient and with much smaller quantities of salt. The amounts of salt used were:

0·19, 0·1, 0·05 and 0·025 g. (*i.e.* 19 %, 10 %, 5 % and 2·5 %) of the weight of faeces. These may be numbered 1–4. An untreated control sample (No. 5) was also used.

The results of this series are of considerable interest. From Nos. 1 and 2 no larvae could be reared and examination of the material at the end of 48 hours showed that all the eggs were dead. From No. 3 larvae were reared up till the end of the third day. None could be obtained on the fourth day, but on the fifth a couple of larvae were hatched out. After this no further larvae could be produced. From No. 4 larvae were produced continuously for five days but not thereafter. On the first two days they were obtained in considerable numbers but on the following three days they were few and feebly developed. From the control numerous vigorous larvae were produced for over a week.

Throughout this experiment it was noticeable that the larvae reared from Nos. 3 and 4 were slow in hatching and developing when compared with the rapid hatching and vigorous growth of those from the control. The only exception was in the case of No. 4 from which, on the second day, a very good brood of larvae was produced. This circumstance may be accounted for by the possibility that the salt had not been mixed in thoroughly and that a small part of the faeces had remained unaffected by it. This would show, as some of the previous experiments also do, that the effect of the salt spreads very slowly and that it is essential that it should be brought into intimate contact with the whole mass of the faecal material.

The larvae in each of these cultures were kept under continuous observation for eighteen days. The larvae in the control cultures pursued a normal course and were fully developed and ensheathed within six days. The larvae in the other cultures, however, had a greatly retarded development. They did not reach their first moult till about the fourth day, and little increase in size could be observed during the next two or three days. Thereafter, however, they began

to develop more rapidly and normally and by the twelfth to the fourteenth day they were fully grown (0·56–0·63 mm. in length) although even then a large number of them did not appear to be ensheathed. The mortality amongst these larvae was greater than normal although not by any means excessive.

This retardation of the development in cultures from 3 and 4 can only be attributed, so far as I can see, to the effect of the salt on the eggs. It occurred to me that perhaps the effect was due to the salt not having been thoroughly washed out in the cultures, but on making accurate estimations it was found that the amount of salt in the culture fluid was only 0·018–0·046 %.

The results of this experiment may be tabulated as follows:

Experiment started 13th April, 1914.

	Weight of faeces	Weight of salt	Percentage of salt	April 14	15	16	17	18	19	20	21
1.	2·1 g.	0·40 g.	19 %	–	–	–	–	–	–	–	–
2.	4·1 g.	0·41 g.	10 %	–	–	–	–	–	–	–	–
3.	4·3 g.	0·21 g.	5 %	+	+	+	–	+	–	–	–
4.	3·9 g.	0·10 g.	2·5 %	+	+	+	+	+	–	–	–
5.	Control			+	+	+	+	+	+	+	+

This experiment appears to show that under favourable circumstances and with thorough admixture of salt even 10 % will effectively kill all the eggs within 24 hours, but that 5 % is not sufficient to kill them in less than five days and is in this respect not much more efficient than $2\frac{1}{2}$ %.

V. This series is chiefly remarkable for the fact that it was conducted with faeces which, as appeared after treatment, contained only eggs of *Necator americanus*. The faecal material was fairly solid but rather moist on the surface. The weights of faeces and salt employed were as follows:

	Weight of faeces	Weight of salt	Percentage of salt
1.	2·22 g.	0·18 g.	8·1 %
2.	1·94	0·12	6·2 %
3.	2·13	0·08	3·8 %
4.	2·85	0·06	2·1 %
5.	2·50	—	Control

As in the previous experiment the salt was thoroughly mixed with the faeces and the technique was the same throughout. Particular

attention was paid to the length of time (in days) required by the larvae to hatch out in each case. The results are given in the subjoined table. (The figures in brackets indicate the number of days required for the eggs to hatch):

May 11	12	13	14	15	16	17	18	19	20	21
1.	+ (4)	+ (3)	–	–	–	–	–	–	–	–
2.	+ (4)	+ (3)	–	–	–	–	–	–	–	–
3.	+ (2)	+ (3)	–	–	–	–	–	–	–	–
4.	+ (2)	+ (1)	+ (2)	+ (3)	+ (3)	+ (5)	–	–	–	–
5.	+ (2)	+ (1)	+ (2)	+ (1)	+ (1)	+ (2)	+ (2)	+ (1)	+ (3)	+ (3)

From this experiment it is obvious that 8 % of salt is not sufficient to kill all the eggs within 48 hours, but it is efficient within three days. It is not, however, any more efficient than 4 %. With 2 % of salt a period of at least six days was required to kill all the eggs or prevent them developing. The control specimen showed that the eggs used in the experiment were capable of developing into active larvae for at least ten days.

With regard to the time of hatching it is evident that in Nos. 1 and 2 the average time was about three and a half days, whereas in the control the time was under two days. There can be little doubt from this that the admixture of salt caused a very marked retardation in the development of the hook-worm eggs.

VI. This series was arranged to contrast the effect of sprinkling as opposed to mixing the salt with the faeces. The results, however, are not satisfactory, apparently because the faeces were not suitable.

The faeces were very moist and contained a moderate number of *Ankylostoma* eggs together with numerous eggs of *Trichiuris trichiura*.

The quantities of faeces and salt used were as follows:

	Weight of faeces	Weight of salt	Percentage of salt	
1.	1·5 g.	0·23 g.	15 %	Salt sprinkled on
2.	2·0	0·16	8 %	,, ,,
3.	1·3	—	0	Control
4.	2·25	0·18	8 %	Salt thoroughly mixed
5.	1·6	0·24	15 %	,, ,,

The results of these experiments were:

July 18	19	20	21	22	23	24
1.	+ (3)	+ (3)	–	–	–	–
2.	+ (3)	+ (3)	–	–	–	–
3.	+ (3)	+ (3)	+ (3)	+ (3)	–	–
4.	+ (3)	+ (4)	–	–	–	–
5.	+ (3)	–	–	–	–	–

It is evident that in the control sample the eggs were unduly long in hatching.

VII. In this series the faecal material was moderately moist and contained numerous hook-worm eggs together with a few larvae. The material had been deposited for some time. Most of the eggs were in an advanced state of development.

This experiment was almost similar to No. 1. The details are as follows:

	Weight of faeces	
1.	2·29	Control
2.	1·7	With 25 c.c. rain water
3.	2·7	With 13·5 c.c. rain water
4.	2·2	With 11 c.c. 10 % salt solution
5.	2·1	With 10 c.c. 5 % salt solution
6.	1·4	With 7 c.c. 2·5 % salt solution

In No. 2 the faeces were covered to a depth of nearly half an inch by the rain water. In No. 3 they were nearly covered, while in 4, 5 and 6 they were a little more than half covered.

The results of these experiments are shown in the subjoined table.

Oct. 31	Nov. 1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.	+	+	+	+	+	+	−	+	+	−	+	−	−	+
2.	+	+	+	+	+	+	+	−	+	−	+	+	−	+
3.	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.	+	+	+	−	−	−	−	−	−	−	−	−	−	−
5.	+	+	+	+	+	+	+	+	−	−	+	−	−	−
6.	+	+	+	+	+	+	+	+	+	+	+	+	−	+

The following notes were made on this experiment:

Nov. 1st. Faeces in No. 2 floated bodily to the surface of the water. Larvae in cultures from Nos. 3 and 4 very scanty.

Nov. 3rd. No. 2 very turbid but larvae cultured on Nov. 1st fairly numerous. On Nos. 3 and 6 a thick grey scum has grown on the surface. On No. 5 a slight broken scum has grown.

Nov. 4th. Nos. 2, 5 and 6 covered with scum. No. 3 overgrown with a thick mould. Of the larvae from the previous day's cultures, they were strong and vigorous in No. 1, few and feebly developed in Nos. 2, 3, 5 and 6 and a few dead in No. 4.

The faecal material and the cultures obtained therefrom were continuously observed throughout. It was thought at first that the overgrowth of mould and scum would spoil the experiment but this did not prove to be the case.

It is evident from the above table that the presence of a moderate amount of water encouraged the development of the hook-worm eggs and larvae, while an amount sufficient to cover the faecal material to a depth of half an inch is not prejudicial. The fact, however, that the faeces floated to the surface in this particular experiment somewhat lowered its value.

With regard to the salt solutions it is evident that 10 % saline is not sufficient to kill the eggs within three days, while 5 % cannot be depended on in less than eleven days. Below that strength salt solution has apparently no more effect than rain water.

It must be remarked, however, that the larvae obtained in these experiments developed much more slowly than those from the normal control. The average hatching time in the control was a little over one day. In the cultures from rain water it was about two days, while in those from salt solution it was generally nearly three days or over.

The control sample became dried up on the seventh day and had to be moistened every second day.

VIII. In this series faecal material from the same patient as in the previous experiment was employed. The quantities used were:

	Weight of faeces	Weight of salt	Percentage of salt
1.	3·0 g.	0·7 g.	23 %
2.	3·1	0·6	20 %
3.	3·6	0·5	14 %
4.	3·3	0·3	9 %
5.	3·9	0·2	5 %
6.	4·3	0	0 (control)

The material was of firm consistency and fairly dry. The salt was mixed up with it.

The results are as follows:

Nov. 1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.	–	–	–	–	–	–	–	–	–	–	–	–	–
2.	+	–	–	–	–	–	–	–	–	–	–	–	–
3.	+	–	–	–	–	–	–	–	–	–	–	–	–
4.	+	+	+	–	–	–	–	–	–	–	–	–	–
5.	+	+	+	+	+	+	+	–	–	–	–	–	–
6.	+	+	+	–	–	+	–	–	–	+	–	–	–

These results are in agreement with those obtained in previous experiments.

IX. In this series material from the same patient as in the two foregoing experiments was used. Three portions were employed.

The first was submerged to the depth of half an inch in rain water, the second to the depth of an inch and the third to the depth of one and a half inches. The experiment was continued for ten days and during all that time larvae were recovered from each of the three lots.

It would thus appear that the submersion of infective faeces in water to the depth of even one and a half inches will not prevent the development of hook-worm larvae.

X. The faecal material used in this experiment was firm but decidedly moist. The salt was merely sprinkled on the surface. The amounts used were as follows:

	Weight of faeces	Weight of salt	Percentage of salt
1.	3·4 g.	0	0 (control)
2.	6·8	0·2 g.	2·9 %
3.	5·1	0·4	7·8 %
4.	4·5	0·8	17·7 %
5.	4·7	1·6	34·0 %
6.	3·4	3·2	94·1 %
7.	5·4	6·4	118·5 %
8.	2·7	12·8	477·0 %

This series is confirmatory of previous experiments.

The results are tabulated below:

Dec. 19	20	21	22	23	24	25	26	27
1.	+	+	+	+	+	+	+	+
2.	+	+	−	−	−	−	−	−
3.	+	−	+	+	−	−	−	−
4.	+	−	−	−	−			
5.	−	−	−	−	−			
6.	−	−	−	−	−			
7.	−	−	−	−	−			
8.	−	−	−	−	−			

Here again it is evident that 17 % of salt sprinkled on the surface is not sufficient to kill hook-worm eggs within 24 hours, and that 8 % is not sufficient to kill in less than four days. In all the cultures larvae were very scanty, in most cases only one or two developing.

XI. *Experiment with sand.*

In this series the material employed was fairly consistent and dry but not formed. Eggs were not numerous. Ten separate portions were used and treated with sand in various ways.

1. Control. No sand.
2. Covered with equal amount of dried sand.
3. Covered with twice amount of dried sand.
4. Thoroughly mixed with half the amount of dried sand.
5. Thoroughly mixed with twice the amount of dried sand.
6. Thoroughly mixed with equal the amount of dried sand.
7. Covered with equal amount of sterilised sand.
8. Covered with half amount of sterilised sand.
9. Thoroughly mixed with equal amount of sterilised sand.
10. Thoroughly mixed with half amount of sterilised sand.

The results of cultures from these experiments are as follows:

Nov.	5	6	7	8	9	10	11	12	13	14	15
1.	+	+	+	+	+	+	+	+	+	+	+
2.	+	+	+	+	+	+	+	+	+	+	+
3.	+	+	+	+	+	−	−	−	−	−	−
4.	+	+	+	+	+	+	+	+	+	+	+
5.	+	+	+	+	+	+	+	+	+	+	+
6.	+	+	+	+	+	+	+	+	+	+	+
7.	+	+	+	+	+	+	+	+	+	+	+
8.	+	+	+	+	+	+	+	+	+	+	+
9.	+	+	+	+	+	+	+	+	+	+	+
10.	+	+	+	+	+	+	+	+	+	+	+

From each of the cultures a considerable number of active and vigorous larvae were obtained.

This series shows that sand, instead of having a deleterious influence on hook-worm eggs and larvae, has exactly the opposite effect, except when the faeces are thoroughly covered by the sand. The experiment, indeed, suggests that admixture with loose sand is an excellent method of rearing larvae for laboratory purposes.

XII. *Experiments on the effect of direct sunlight.*

As a preliminary to this series a small experiment was performed to test the effect of exposing the faecal material. Some faeces were freely exposed on a plate on the laboratory bench with doors and windows wide open during the day time. Another portion of faeces was exposed

in a similar manner with the exception that it was first sprayed with water for five minutes and the process repeated every 24 hours, the surplus water being drained off. Two control samples were kept covered.

The first and second portions yielded larvae for two days but none thereafter. The controls produced larvae for over a week when the experiment was discontinued.

This appears to show that in the natural course of events when the faecal material is freely exposed in the absence of moisture the hookworm eggs may die off within three days and that the moistening of the material every 24 hours does not prolong the life of the eggs.

XIII. The next series was intended to test the effect of direct sunlight. The faeces were exposed under various conditions to the direct rays of the sun, and for the purpose of comparison other portions of faeces were exposed under similar conditions in the shade, out of doors and in the laboratory. The material used was solid and fairly dry.

The various portions were placed as follows:

1.	On paper	Exposed to direct sunlight in the open air.
2.	In covered Petri dishes	
3.	In open Petri dishes surrounded with water	
4.	In covered Petri dishes surrounded with water	
5.	In open Petri dishes	Outside in the shade.
6.	In covered Petri dishes	
7.	In open Petri dishes surrounded with water	
8.	In open Petri dishes	In room in the shade.
9.	In covered Petri dishes	
10.	In open Petri dishes surrounded with water	

The experiment was started at 4 p.m. when the shade temperature was 95° F., but during the course of the experiment it fell to 90·5° F. Earlier in the day the temperature had reached a maximum of 104·2°. The wet bulb temperature rose during the experiment from 78·6° to 80·6° F. The solar radiation registered 154·5° F. at 3 p.m. During the whole course of the experiment the sun continued to shine brightly.

Cultures were made from Nos. 5–7 at 5 p.m. and again at 6 p.m.

The results were as follows:

	1	2	3	4	5	6	7
5 p.m.	+	−	+	−	+	+	+
6 p.m.	+	−	+	−	+	+	+

The experimental plates were then removed indoors and kept there for nearly two days.

At 2 p.m. on the second day cultures were again made with the following results:

1	2	3	4	5	6	7	8	9	10
−	−	+	−	−	+	−	+	+	+

This experiment provides one or two rather interesting conclusions. It shows that hook-worm eggs can resist the effect of strong sunlight for over two hours when the faecal material is freely exposed either alone or surrounded by water. When, however, the material is exposed *under glass* to the direct rays of the sun, the eggs are all killed off within an hour whether there be water present or not. It seems very probable that this result is due to the heat concentrated within the covered glass dish. On the other hand in the case of the material placed in the shade the eggs in the covered specimen survived longer than those in the uncovered specimens.

XIV. This series was similar to the last with certain modifications. The material used was solid and slightly moist. The procedure adopted was as follows:

1. Part placed in covered glass dish
2. Part placed in covered glass dish with water
3. Part spread out thinly in covered dish
4. Part spread out thinly in covered dish with water
5. Part placed in open glass dish
6. Part spread out thinly in open dish
7. Part placed in open dish with water
8. Part spread out thinly in open dish with water

} Exposed to direct sunlight from noon till 1.30 p.m.

9–16. Duplicate series—exposed to sunlight

17–24. Control series—kept in room

The average shade temperature registered during the experiment was 83·2° F. with a wet bulb of 73·4° F. and a solar radiation up to 1.30 p.m. of 138·2°. Indoors the temperature was about 81·5° F.

After an exposure to the sunlight the plates were removed indoors and water was added to all except Nos. 1, 3, 17 and 19. They were then kept in the room for ten hours. Cultures were made daily for the following four days.

The following table shows the result.

	26/4	27/4	28/4	29/4
1.	−	−	−	−
2.	−	−	−	−
3.	−	−	−	−
4.	−	−	−	−
5.	+	−	−	−
6.	−	−	−	−
7.	+	+	+	+
8.	+	+	+	+
9.	−	−	−	−
10.	+	+	+	+
11.	−	−	−	−
12.	−	−	−	−
13.	−	−	−	−
14.	−	−	−	−
15.	−	−	+	+
16.	+	+	+	+
17.	−	−	+	+
18.	+	+	+	+
19.	−	−	−	−
20.	−	−	−	+
21.	+	+	+	+
22.	+	+	+	+
23.	+	+	+	+
24.	−	−	+	+

The results of the first two series may be combined in the subjoined table.

	26/4	27/4	28/4	29/4
1.	−	−	−	−
2.	±	±	±	±
3.	−	−	−	−
4.	−	−	−	−
5.	±	−	−	−
6.	−	−	−	−
7.	±	±	+	+
8.	+	+	+	+

The chief discordant result here is in the case of No. 2 in which the faeces were surrounded with water and exposed in a covered dish. Possibly an exposure of $1\frac{1}{2}$ hours is not always sufficient to kill all the eggs in this case.

In this experiment the temperature was about 10° F. lower than in the preceding one.

XV. The last experiment was intended to be a duplicate of the previous series. The faecal material was duly obtained in the early morning. The plates were prepared as before, but the sun remained overcast during the whole day. The plates were, therefore, covered and kept in the laboratory. Next day the sky was again heavily overcast and there was practically no bright sunshine all day. For that reason the experiment was again delayed.

On the following day the clouds had somewhat dispersed and after midday the sun shone at fitful intervals. Two lots of material were exposed as before from 12.15 p.m. to 4.15 p.m. while a third lot was kept in the room. During this time the mean shade temperature was 76·0° F. with a wet bulb of 67·6° F. The maximum solar radiation was 121·0° F. while the indoor temperature was 76·6° F.

Cultures were made from all the plates at 4.15 p.m. and on each of the following four days. With few exceptions all the cultures produced numerous vigorous larvae.

The results of this experiment do not appear to show any points of importance. It is evident that the interval of 48 hours at room temperature was sufficient to enable the eggs to hatch and the larvae to develop, and that the subsequent exposure to the open air for 4 hours was not sufficient to arrest the development. It may be noted, however, that a certain proportion of the larvae from the exposed dishes were inactive, though not dead.

Summary and Conclusions.

1. There can be no doubt that Ankylostomiasis has obtained a very firm hold in the coastal districts of North Queensland, and every effort should be made to arrest its spread, and if possible eradicate it.

2. Experience in other parts of the world, notably the United States and Germany, has demonstrated the great difficulty of coping with the disease, even with the most energetic of preventive measures.

3. The disease gives rise to much sickness and inefficiency especially amongst children, and although competent medical treatment is usually efficacious the most satisfactory and permanent results are to be expected in the direction of prevention.

4. The chief preventive measures are individual cleanliness and thorough and rapid destruction of night soil and deposits of faecal material.

5. In the presence of a properly organised sanitary system, intelligently utilised, there should be little or no risk of infection. It is the promiscuous distribution of excreta that is the chief source of the spread of infection.

6. If the indiscriminate deposit of faecal material be not prevented, the matter resolves itself into a problem of considerable difficulty.

7. The more commonly used disinfectants, if thoroughly employed, would render the faecal material comparatively innocuous, but their use is not less laborious than the proper removal of the faecal material and its disposal in properly constructed receptacles.

8. Common table salt has a decidedly injurious effect upon the hook-worm eggs but it requires to be brought into very intimate contact with the infected material. The process of merely sprinkling the surface is almost futile unless the salt be used in enormous quantities.

9. When mixed with faecal matter, sand promotes the development of hook-worm larvae, but when used as a covering of a certain depth it arrests development.

10. Exposure to direct sunlight of sufficient intensity kills hook worm eggs and larvae very rapidly.

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NOTES ON THE MORPHOLOGY OF CHALCIDOIDEA BRED FROM CALLIPHORA.

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(With 2 Text-figures.)

Melittobia acasta Walker.

Melittobia acasta, Walker (*Cirrospilus acasta*), *Mon. Chalcid.*, Vol. I, p. 328, ♀ (non ♂) (1839).

Syn. *M. Audouini,* Westwood, *Proc. Ent. Soc. Lond.,* Vol. v, Pt 2, p. xviii (1848).

Syn. *Anthophorabia retusa,* Newport, *The Gard. Chronicle,* p. 183 (1849).

Syn. *Anthophorabia fasciata,* Newport, *Trans. Linn. Soc.,* Lond., Vol. XXI, p. 81, ♂, ♀, pl. 8, f. 4–6 (1852).

Introductory Notes.

This interesting and peculiar parasite was apparently first noted and studied by George Newport in 1831. That writer, however, was unable to publish his observations till 1849, by which date he had been anticipated in authorship by Francis Walker for the specific name, and by J. O. Westwood for the generic. With the latter Newport engaged in a somewhat protracted controversy touching priority of name, morphology, habits, etc., of *Melittobia.* There can be no doubt that Westwood's genus, very briefly characterised in *Proc. Ent. Soc. Lond.* for 5th July, 1847, p. xviii, must stand. The part of the proceedings containing this page was published on 12th January, 1848. As the purpose of the present notes is chiefly to offer a more accurate description of *M. acasta,* and as, besides, I am at present engaged in revising the whole genus, it does not seem necessary to say much on the habits of the insect. *M. acasta* is markedly polyphagous; never

apparently a true hyperparasite, it attacks everything within its limited range of action. Already it has been bred from a long list of hosts, and somewhat contradictory conclusions about the insect's parasitic status and economic value may be drawn from its host attachment. Thus it may be a pest in nests of bees, wasps, etc.; a species of *Melittobia* again proved a serious menace in the laboratory to Tachinids introduced into the U.S.A. to combat the Gipsy Moth, but possibly, the insect plays, or may be induced to play, a useful part in destroying dipterous puparia in or near houses, or in surroundings where the adult may skulk secure. For *Melittobia* dislikes light, and the ♀ flies little, though when once established in a suitable environment, it is hard to dislodge.

In the following notes special attention is drawn to the chaetotaxy, particularly of the legs. In the ♂ the much modified antennae, and probably the curious fore tarsus and long bristles of the mid legs are accessory organs of copulation. The ♂♂ are inveterate fighters, and the greater development of the mandibles, which in both sexes are powerful, seems to be connected with this habit. The extraordinary sequel to the arrhenotokic parthenogenesis exhibited by *Melittobia* has been independently noted by Fiske (1911) in America and Malyshev (1911) in Russia.

The ♂♂ of this genus are conspicuous; the ♀♀, however, are not so easily recognised. The face, mandibles, prothorax, postero-laterally alate metathorax, and the propodeon (a little flattened in Fig. 1, *d*, to show the pleural bristles), give reliable characters. The ♂ is at first of a transparent yellowish-brown colour, the head sometimes darker, but after feeding, the abdomen may be opaque. The general colour also darkens with age. The ♀ is blackish, or blackish-brown, with a dull, metallic, coppery lustre; the tarsi, apices of the femora and tibiae paler, the latter infuscated near, or with a smoky streak from, the base. The size of the species varies presumably with nourishment. The ♀ is from $1\frac{1}{2}$ to $2\frac{1}{4}$ mm. in length; the ♂ owing to the shorter abdomen is a little less, but one of Dr Graham Smith's examples of this sex was well over 2 mm. The alar expanse of the ♀ varies from $2\frac{1}{4}$ to $2\frac{1}{2}$ mm.; of the ♂, about $1\frac{1}{4}$ mm. Walker took *M. acasta* "on windows," a rather suggestive reference in the present connection. I have seen examples from Oxford (Hamm) and Cambridge (Graham Smith); and from Sir Sidney Saunder's collection I have mounted and examined material from Albania and Corfu. Except that in the latter lots the ♀ antennae are a little more slender, the head (♀) possibly narrower, and similar minor differences, I can see nothing to justify the belief

that we have more than one European species, and I venture to think that *M. osmiae*, Thoms., ♂, 1878 (*Hym. Scandin.*, Vol. v, p. 204), is merely a synonym of *M. acasta*.

In the figure of the radius (Fig. 1, *e*) the dotted hair is present in a number of specimens. The pigmentation of the rudimentary lateral eyes of the ♂ is often, in life at least, quite pronounced. The figure of the ♂ antenna (Fig. 2, *c*) is designed to show the inward bend of the funicle from the second joint (dotted) onwards.

Melittobia acasta Walker (1839).

♀. *Head.* Longer than broad (14 : 13); eyes, $\frac{1}{2}$ of the total depth, separated by 3 diameters across the middle of the frons; in profile small. Vertex extending distinctly above the eyes while below the malar space is nearly $\frac{1}{5}$ of the eye's depth. Malar keel marked, forming with the clypeal edge a rounded angle. Two large rounded median clypeal lobes not widely separated. Ocelli nearer to one another than to the orbits, the anterior one well above the eyes.

Reticulation of vertex and frons consisting of rather large feebly raised cells, which are more elongate on genae and occiput. Scrobes small, quadrate, corners rounded, narrower superiorly, set far down on the face, halfway between base line of the eyes and mouth edge, and rather wide apart. There is a nearly smooth broad triangular supra-clypeal area contracting to its narrowest in the middle of the frons just above the base line of the eye, from which point the smooth surface continues upwards as a narrow groove whose sides diverge very gradually towards the anterior ocellus. Eyes practically bare, not more than a few very short scattered bristles being visible under high magnification, 8–10 short bristles along each orbit. Face and vertex with numerous bristles—about 40 (not counting the orbital bristles) on each side of the median depression, 8 (4, 4) between the scrobes and 2–3 on each clypeal lobe.

Antennae. Length 0·45 mm., scape, pedicel, ring-joint (compound), 3 in funicle, 3 in club. *Scape* (4 : 1 at apex) narrow at base and gradually expanded, about as long as the three normal funicular joints and the first segment of the club together, many short external bristles, 9–12 crossing the ventral edge, and about the same dorsally. On inner apical half 8–10 bristles, and 2 stronger subapically above the hollow for the reception of the pedicel. Pedicel (5 : 3) $\frac{5}{12}$ of the scape. Ring joint narrow, a little more than $\frac{1}{2}$ the width of the pedicel, with 2 joints each of which consists of 2 closely appressed laminae, whose distinctness can be demonstrated by pressure. Funicular joints subequal (14 : 14 : 15) increasing in width (15 : 17 : 18). The club (11 : 5) (as long or nearly so as the 3 normal funicular joints together) segmented in ratio 17 : 14 : 13, with terminal spur giving rise to a spine. The short "mushroom"-like truncated bristles of the funicle, 1–2 on each joint, rise from large pustules. Sensoria few, broad, moderately raised but without long points, except on the 3rd division of the club, arranged as follows (*a*) 3, (*b*) 4, (*c*) 4, (*d*) club 4–5 : 4–6 : 3.

Mouth parts. Mandible somewhat long, tridentate, the outermost (lowest) and middle teeth acute, the former longer; the third tooth small, rounded—

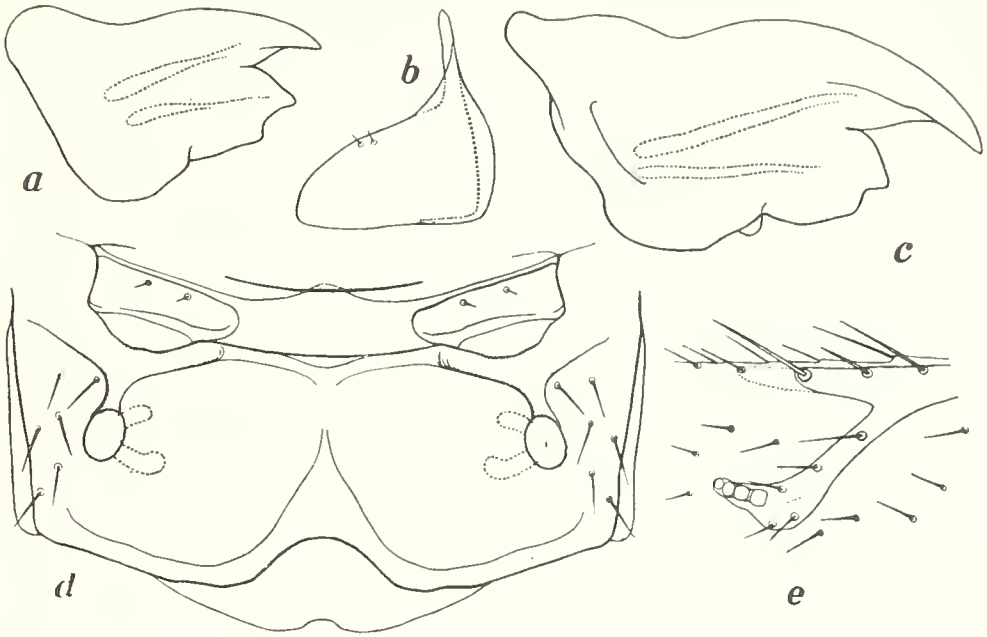

Fig. 1. *a*, right mandible, ♀; *b*, prepectus, ♀; *c*, right mandible, ♂; *d*, metanotum and propodeon, ♂; *e*, radius and postmarginal, ♀.

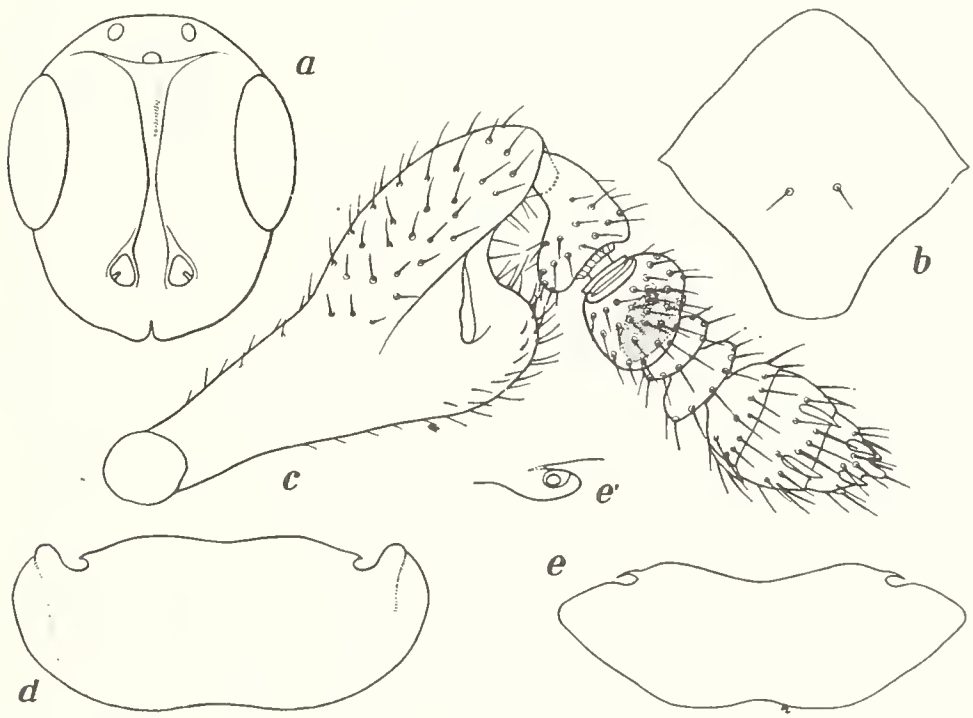

Fig. 2. *a*, head, ♀; *b*, prosternum, ♂; *c*, right antenna (outside), ♂; *d*, pronotum, ♂ (nearly flattened); *e*, pronotum, ♀ (completely flattened); *e'*, spiracular notch, ♀.

rectangular. The two internal hollows equal. Lower edge appreciably concave on proximal half. About 12 external bristles arranged mainly in 2 transverse rows. *Trophi*, maxilla, cardo much narrowed above the "foot" but broadly based on the stipes, the latter long with large faint pattern, the lateral bristle long, hardly shorter than the palpus which bears 4 stout bristles, the first beyond $\frac{1}{2}$, and the rest (one long) apical or subapical. There is also a minute bristle between the base of the palpus and the inner edge of the stipes—nearer the latter. Galea 4–5 short thick bristles on large pustules and there are 8–10 longer and thinner on inner (upper surface). Sub-mental sclerite distinct, mentum with 1 long bristle well before $\frac{1}{2}$. Labial palpus (a little over $\frac{1}{2}$ of the maxillary) with 4 bristles (one longer) apical or subapical. Lingua with 4 setigerous cells—the setae unusually long, flat and stout.

Thorax. Prothorax porrect, pronotum however transverse in one tergite, slightly sinuated antero-medianly and concave posteriorly. Spiracles embedded and half surrounded behind by a downward projection of the sclerite. Surface raised reticulate, the pattern medianly smaller and more regular, cells larger towards and on the overlaps. The latter bare as is also (very narrowly) the middle of the protergite. Posterior row of 12 longer bristles (6, 6) and about 60 shorter (30, 30). Pre-episternite long, reticulate, 6–9 short bristles. Prosternum quadrate, truncate behind, reticulation feeble, moderate. 2 bristles postero lateral (1 : 1). *Mesothorax*, mesonotum mid lobe bare anteriorly with about 40 (20 : 20) short bristles on the posterior $\frac{2}{3}$ and 1 longer at each presutural angle, side lobes 12–14 bristles, 1 longer before the axillae, the latter bare, tegulae 2 bristles. Scutellum, 2 sulci distinct (besides the lines separating dorsum from pleurae) and the non-setigerous pustule (at $\frac{1}{3}$ from posterior edge) is nearly aligned with the anterior and posterior bristles. Except just behind the suture and there narrowly, the pattern of the scutellum is fine striate. Elsewhere the mesonotum shows a somewhat coarse but hardly raised reticulation, the cells, longer than wide for the most part, reach their greatest elongation in the middle of the mid lobe. Mesosternum smooth, with four bristles (1, 2, 1) at the posterior suture. Prepectus produced narrowly for some distance at the anterior angle, reticulate, with 2 bristles on the anterior edge at $\frac{1}{2}$. The pattern of the prepectus extends backwards behind the suture on to the mesopleurae which however are for the most part smooth and bare. Metathorax, postscutellum anteriorly obtuse-angled, posteriorly convex without median keel, bare, with large delicate pattern. The side pieces (each with 2 bristles) smooth except narrowly towards the postscutellum, broaden out convexly posteriorly in front of the spiracular sulcus. *Propodeon*—sides a little convergent posteriorly. Hind edge roundly incised for the petiole. Spiracle moderate sized, oval, nearly circular. The dorsum with median incrassation fading anteriorly, hardly carinate. Surface reticulate, the median cells equal, those round the spiracle compressed and elongate. The sulcus from the spiracle sends one branch towards the base of the hind wing and another (narrow) towards the middle of the notum. Pattern of metapleurae coarser and distinctly raised, with 6 bristles (one of them minute) at the anterior edge of the spiracle.

Wings. Fore wings, $2\frac{1}{2}$ times as long as broad, length 1 mm., breadth 4 mm. Submarginal : marginal : radius : postmarginal 11 : 11 : 3 : 3. The radius, rather

acutely inclined to the postmarginal, expands gradually to its obliquely truncated apex. Up to the 2 clear cells at the junction of the marginal and the submarginal there are 6–7 bristles, 4–5 major, 1–2 at base of marginal minute; on the marginal stand, 10–11 overlapping bristles, while on the radius are 5–6, mostly towards the apex. The postmarginal has 3–4. 13–15 bristles in the submarginal cell. The discal ciliation covers the entire surface extending even over the basal triangle. There is however, before a row of cilia from the base to the discal end of the frenulum, an extremely narrow bare line; marginal fringe short. *Hind wings* about four times as long as broad, length 8 mm., breadth (including cilia) 0·2 mm. Submarginal and marginal equal. Submarginal with two isolated bristles near base—otherwise bare, marginal 6–7 widely separated bristles. Comb of retinaculum with only 5 minute bristles. Discal ciliation regular, no bare line, the basal triangle nearly bare, marginal fringe long and sparse.

Legs. *Fore legs*, coxae (11 : 5), rather elongate, $\frac{7}{9}$ of femur, coarsely reticulate, 5–6 bristles in anterior median perpendicular row, 1 bristle (stout) at the inner apical angle and 6–7 more posteriorly on the apical half. Femur a little swollen medianly. Anteriorly sparsely set with bristles which form no definite ventral row though 6–7 cross the ventral edge. Subventral row (6–7 bristles) with two others less regular above. Posterior surface like anterior but with fewer bristles. Tibia anteriorly about 9 transverse rows of bristles, 3 in a row. Apical comb of 5 spines. Posteriorly nearly bare, one row of bristles (8–9) ventral on basal $\frac{2}{3}$ and sub-ventral apically, where there are 3–4 extra bristles below. Apical spur longer than 1st tarsal joint. Tarsus. I. Anteriorly 3 short stout bristles in a row, rising from clear pustules, represent the usual lateral comb. 3 stout spinose bristles at upper apical angle. II–III, like I with an extra mid-dorsal bristle. Claws gradually tapered and straight to near the tip. *Mid legs*, coxae (3 : 2) oblong, rounded postero-ventrally, externally reticulate and nearly bare. 2 bristles above the trochanter and 2–3 near the posterior edge and 1–2 round apex. Femur (4 : 1), the "head" sharply constricted off below. Anterior bristles subdorsal row 9, 2 subventral behind the constriction and 2 more apical and median, on ventral edge 4–5 short bristles (the row continued near the apex by 1–2 more rising *anteriorly*). The usual subapical pair of stronger bristles are present. Posterior bristles, subdorsal row 6–8, 3 more medianly on apical $\frac{1}{3}$. Tibia, narrow, not expanded, anterior bristles 2 rows (10–12 each). Posteriorly median row 8–9 the last 3 with an extra bristle towards the ventral edge. Apical spur longer (7 : 6) than 1st tarsal joint. *Hind legs*, coxae a little swollen behind superiorly, 8–10 external bristles. Tibia slightly expanded towards the apex where there is a distinct anterior comb of spines (5) as well as the usual posterior one (9–11).

Proportions of tarsal joints (excluding claw).

	I.	II.	III.	IV.
1	12	18	15	27
2	21	24	18	27
3	25	27	20	30

Abdomen. Dorsal surfaee smooth exeept narrowly behind the petiole and extensively on the overlaps of the tergites. Reticulation a little more pronouneed between the widely separated spiracles and between the stylets. Tergites subequal, 3 and 4 longer. Bristles short and fine. Tergite i bare on the disc, 5–6 bristles on eaeh overlap. Tergite ii with similar lateral bristles and a widely interrupted posterior row (4–5 : 4–5). Tergites iii and iv have 2 irregular rows post-median and posterior, there being 7–8 in the first on eaeh side and a few more in the seeond. (Tergite iv shows 24 bristles in the posterior row.) On the 5th tergite there are traces (3–4 bristles on eaeh side) of a 3rd row in front of the median one while in the posterior row (16) 3–4 at the sides are stronger. The spiracles are small, nearly round with 7–9 bristles behind eaeh and 16 in a row between. 12–14 between the stylets. Ovipositor, free portion of the sheath $\frac{2}{7}$ of base which bears 3–4 bristles on apieal $\frac{1}{3}$. Only the apieal $\frac{1}{6}$ of the needle is serrate, the teeth minute.

♂. *Head.* From in front rounder and broader than in the ♀, very narrowly flattened on the mid vertex. Clypeal lobes more widely separated. Eyes atrophied, the eorneal area faeetless, but when more closely examined rudiments of the internal "cup" or selerosed rims ean be made out. The whole head nearly uniformly ehitinised, definite plates and sutures almost unrecognisable. Lateral oeelli smaller than the anterior—the three forming a small triangle on the top of the swollen vertex. Serobes broader and nearly eircular. No X-shaped postseapal hollow. Reticulation or pattern where traeeable finer and extremely faint, on vertex and occiput obsolete or nearly so; on genae and round the serobes a few delieate striae oeeasionally intereonneeted ean be made out. Numerous bristles on vertex (more sparingly) and face, about 60 on eaeh side of mid line between anterior ocellus and scrobes. Round the inner margin of each scrobe are 6–8 bristles; 3 on eaeh median clypeal lobe before the edge, 1 at the edge, and 1 (fine) from below. Below the scrobes 6–8 bristles, and 6–8 along the mouth edge from below.

Antennae. Scape, pedicel, ring-joint, funiele (4), elub (3); length 0·4 mm. Seape (2 : 1) enormously developed, triangular, upper distal angle narrower and produeed beyond the wider ventral one with a broad and deep semicireular eoneavity between. The inner edge of this hollow is set with convergent bristles. Outer surfaee of scape flatter and barer but with numerous short bristles on the upper apieal half and a few ventrally; inner more swollen espeeially towards the apex with evenly but sparsely set bristles. Within the apieal exeavation there is a clear area narrowed towards the base of the pedieel and broader, more oval, ventrally. Pedicel (6 : 5) triangular, ventral angle rounded, ventral edge excavated, 9–12 bristles externally. Ring-joint possibly as in ♀ but I have been able to distinguish only (*a*) a basal stalked joint with a flange (deeper below than above) and (*b*) and (*c*) 2 laminae. The flange of (*a*) is perhaps bilaminate, (*b*) and (*c*) are elosely applied to the mueh swollen 1st normal funicular joint. The latter as wide as the elub and $\frac{1}{3}$ wider than the 2nd joint so mueh more developed on outer aspect that the other joints and club are inwardly displaced and the funicles beeome medianly eonvergent. The remaining 3 funicular joints short, transverse, distally straight edges, posteriorly

globose, with a whorl of 10–12 bristles, the 3rd and 4th also sometimes with 1 sensorium each. The club, much swollen, $\frac{1}{3}$ as wide again as last funicular joint, 3 jointed, the last with a short spur, possesses sensoria (*a*) 3, (*b*) 4, (*c*) 2. Mouth parts essentially as in the ♀. Mandibles narrower, the outer tooth stronger and a little more incurved.

Thorax. Prothorax largely developed, quadrate, pronotum longer than the mid lobe of the mesonotum. Posterior edge straighter than in the ♀. Overlap in the ♂ oblong while in the ♀ more cut away from the anterior to the postero-lateral angle. Prosternum practically square, 2 minute median bristles. Episternite with 10–12 bristles. Parapsidal furrows showing a distinct chitinised folding only shortly anteriorly, but continued back to the suture as a broad pale blaze. Chaetotaxy of mesonotum as in the ♀, with fewer bristles on mid lobe, scutellum without impressed lines and showing only two pairs of setigerous pustules, (*a*) at $\frac{1}{2}$, and (*b*) on posterior margin. Pattern of ♂ thorax all over finer and fainter. Sternopleural surfaces—including even the prepectus—smooth. Metathorax and propodeon as in ♀, smooth except the lower pleurae where the bristles are stronger and all equal. Internal median propodeal thickening more pronounced in ♂.

Wings. Narrowed and reduced on apical $\frac{1}{2}$. *Fore wings* (3 : 1), length 0·54 mm., breadth 0·18 mm. Long, oval, contracted a little near base, anterior and posterior margins subparallel, submarginal : marginal : radius 5 : 5 : 1. The postmarginal rudimentary. Submarginal cell large, with 6–8 bristles, submarginal vein 4 bristles, marginal 3 bristles at origin and 6 stouter overlapping the costa with the same number (finer) from below. Radius—3 on vein and 1–2 on head which has 3 cells. Basal triangle up to origin of marginal vein bare. Thence, parallel and near to hind margin, extending to end of frenulum, is a row of bristles with a broad bare line anteriorly. Discal ciliation dense and stout, fringe fine, very short. *Hind wings* (4 : 1), length 0·37 mm., breadth 0·9 mm. Besides the 2 basal bristles the vein bears 1 stronger and 1 weaker before the 2 clear cells. Retinaculum weak. Fringe longer and stouter than in fore wings.

Legs. *Fore legs*, coxae (9 : 5) large, swollen, $\frac{2}{5}$ of the femur, pattern weak and obscure except externally on posterior surface where the cells are large. 8–9 short weak bristles in 3 transverse rows and 2–3 longer apically—all on outer anterior surface. Inner surface bare, faintly reticulate. Femur (3 : 1) swollen beyond $\frac{1}{2}$, lower edge straight, anteriorly set evenly with bristles, no ventral row. Fewer bristles posteriorly and chiefly on apical $\frac{1}{2}$. Of these 5–6, median subapical, are stronger. Tibia (5 : 1) as long as femur, swollen at about $\frac{1}{2}$, with similar bristles to femur, but barer posteriorly—only 6–8 (short) in a subdorsal row which curves inwards at apex where the irregular comb consists of 6–8 spines. Tarsal joints remarkably bare, 3 and 4 fused but not quite completely, there being an internal S-shaped incrassation at the union of the joints. The 1st joint bears anteriorly 3 stout ventral bristles, 1 in the middle of the sloped apical edge, and 1 (finer) at the dorsal apical angle. 3 fine postero-ventral spines; 2nd joint like the 1st but with 1 spine anteriorly and another posteriorly. The fused joints repeat the arrangement of the second. *Mid legs*, coxae large, swollen, with surface externally shagreened or scaly reticulate—

the cell outlines raised chiefly ventrally. 3 stout anterior bristles in a vertical row below $\frac{1}{2}$ and 3–4 minute posteriorly above the trochanter and about the same number on the posterior edge apically. Trochanter prominent, in side view helm-shaped, broadened before the femur, with a patch of long bristles (about 10) towards the apex anteriorly and 3–4 more in a ventral row; posteriorly are only 2–4 minute bristles. Femur (3 : 1) triangular in cross section, with 3 surfaces, antero-dorsal, antero-ventral, and posterior. Mainly along the blunt edge between the anterior surfaces, from base to apex, is a broad belt of bristles (2–3 deep) which up to $\frac{3}{5}$ are fine rising inconspicuously. Towards and at the apex the bristles, much longer (nearly half the length of the tibia) and thicker, rise from large pustules. Above this belt the femur is extensively bare, 8–9 subdorsal bristles, in 2 irregular rows, on apical $\frac{1}{2}$, over dorsal edge 14–16 short bristles appear. Antero-ventral surface below the belt bare; postero-ventrally a minute subapical tooth; ventral row 6–8 fine bristles. Posteriorly the femur is nearly bare; 15–16 short bristles in 2 subdorsal rows beginning before $\frac{1}{2}$; 7–8 more on apical $\frac{1}{4}$ of which 2–3 (subapical) are stronger. Tibia (3 : 1) narrowed on basal $\frac{1}{5}$; much swollen dorso-ventrally; distinctly shorter (17 : 20) than the femur and rather heavily chitinised dorsally. Anterior subdorsal row 9–11 bristles; 5–6 more towards apex where there is an irregular transverse row 3–4. Tibial spur exceeding 1st tarsal joint. *Hind legs*, coxae (4 : 3) 4 short apical bristles anteriorly and 1 (stouter) subapically on ridge above trochanter. Posteriorly about a dozen bristles on apical half. Femur (rather over 3 : 1) anteriorly bare subdorsally to the middle from base to near apex, elsewhere covered with bristles not closely set; posteriorly 11–13 bristles in 2 subdorsal rows; 15–17 in median and sub-median rows; ventral row 5–7. Tibia barely longer than femur, evenly set with bristles on both aspects but barer on antero-basal quarter. Besides the normal (posterior) apical comb (14 spines) there is an irregular anterior comb of about 9 spines. Apical spur as long as 1st joint of tarsus. The tarsal joints being longer have a few extra bristles, *e.g.* on I there are 3 stout lateral above the antero-ventral spines, and 2–3 on the same surface elsewhere; 5 postero-ventral spines with 2 above; a pair of dorsal bristles behind the apical pair.

Proportions of tarsal joints.

	I.	II.	III.	IV.
1	15	17	40	
2	25	22	15	30
3	40	32	24	42

Abdomen. Smooth but the posterior tergites tend to be raised in minute points near their hind margins. Spiracles small, stylets with 10 bristles (5 : 5) between and a patch of about 12 outside each, of these 4 near the stylets are stronger.

Genitalia. Tooth of the clasper very short.

A NEW AFRICAN LOUSE (*POLYPLAX CALVA* N. SP.) FROM *CRICETOMYS*.

By JAMES WATERSTON, B.D., B.Sc.

Imperial Bureau of Entomology, London.

(With 2 Text-figures.)

Polyplax calva sp. nov.

♂♀. *Head* (Fig. 2, *A* and *B*) long with well-marked postero-lateral angles, and distinctly angled posteriorly; bare above, behind the pre-antennal suture, except for two postero-lateral spines (1, 1). *Antennae* similar, the first joint of the ♂ larger and broader. *Tergites* and *sternites* indistinctly developed, consisting of small discontinuous chitinous areas at the base of the bristle; *pleurites* reduced, projecting shortly above and below in minutely denticulate or frayed points.

♀. *Head* (5 : 3) produced before the antennae about the length of the second antennal joint, and, as seen in spirit specimens, a little truncate anteriorly; when mounted in balsam more pointed owing to the flattening out of the conical mouth opening; the latter with about six minute bristles and ten denticles. The narrow pre-antennal portion of the head bears dorsally and anteriorly two more approximated, and posteriorly two more widely separated minute bristles; two similar bristles at the side; antero-ventrally there are two short bristles behind the perioral chitinous ring, and there is the usual pair of bristles (1 : 1) just at the insertion of the antennae. Behind the antennae the head has the sides subparallel, very little swollen and remarkably bare both ventrally and dorsally; the postero-lateral spines almost half the greatest breadth of the head. *Antennae*: the first and third joints, also the fourth and fifth, are equal; the third joint is longest. The first joint is one-fourth broader than long; all the others longer than broad, and the second, third and fourth are about the same thickness; the fourth joint is square, and the fifth half as long again as broad. The sense organs on joints 4–5 are small and circular; on the fourth joint single; on the fifth double and fused.

Thorax: one minute spine above the spiracle of the prothorax; mesothoracic spiracle with one stouter short spine in front and a long, very stout bristle behind, otherwise bare dorsally. *Sternal plate* shield-shaped, seven-sided (see Fig. 2, *G*), and in well-marked specimens with a faint, straight prolongation between the anterior (first pair) coxae.

Legs. The fore and mid legs are of the same length and calibre, the only difference being that in the latter the tibia is about one-eighth broader apically and the claw a little longer, stouter and darker. Their chaetotaxy, too, is practically identical, the femur bearing 3–4 minute dorsal bristles, and 3–4 short spines anteriorly. The tibia with four ventral short spines towards the apex, and two equal subventral, one near the apex, and another before one-half, posteriorly; anteriorly there are also two spines, that near the apex (almost median, not subventral) more robust, the median one much smaller. Mid coxa larger than fore coxa; hind legs (Fig. 2, *C* and *D*), claw short and blunt.

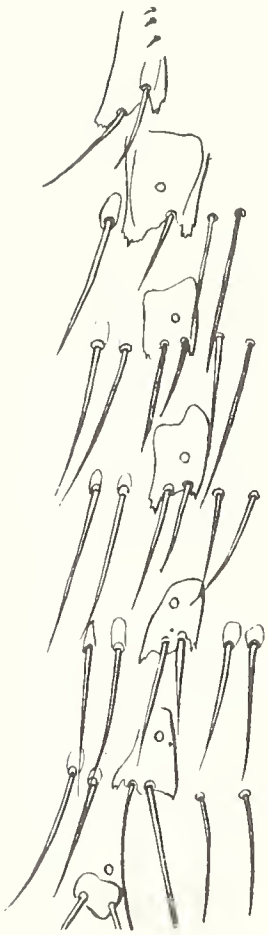

Fig. 1. *Polyplax calva*, n. sp. ♀ (Accra). Pleura, Pleurites and chaetotaxy.

Abdomen. The slight development of the sclerites in this region and the consequent obscuring of the segmentation makes it difficult to assign to their respective positions the various rows of bristles, etc. Of these rows there are, on the dorsal surface, about fifteen, arranged as follows:

	I. and II.	III.	IV.	V.
Tergites	2, 4, 7	7	6, 7	6, 8
*Pleurae and Pleurites	2	1–1–2	2–2–2	2–2–2
Sternites	5, 4	7, 6	7, 6	7, 6

	VI.	VII.	VIII.	IX.
Tergites	6, 7	8, 7	5, 7	4
*Pleurae and Pleurites	2–2–2	2–2†–2	2†	2
Sternites	7, 6	6, 6	2	—

The edge of the genital plate, which is posteriorly gently concave with a blunt angle medianly, is frayed with about sixty short spine-like processes. Gonopods short, rounded, wide apart, with one short stout spine inwardly directed at the inner angle, and four along the edge outwardly. The tenth sternite bears on each lobe two minute bristles.

Dimensions of P. calva, ♀.

	Length	Breadth
Head	0·35	0·2
Thorax	0·2	0·35
Abdomen	1·2	0·65

Total length 1·75 mm.

* See Fig. 1.

† These bristles are much longer than the others.

Antennae. Length 0·23 mm.

	Length	Breadth
1	0·048	0·060
2	0·055	0·040
3	0·050	0·038
4	0·038	0·036
5	0·038	0·024

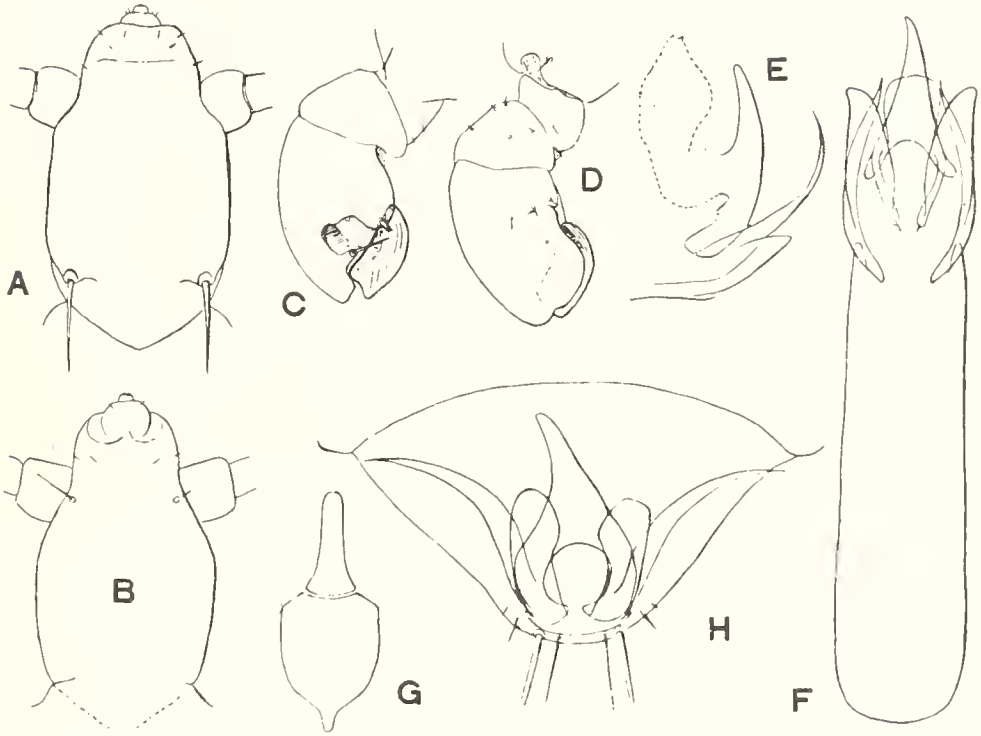

Fig. 2. *Polyplax calva*, n. sp. ♀ (Accra). *A*, head (upper surface); *B*, head (under surface); *C*, hind leg (posterior surface); *D*, hind leg (anterior surface); *G*, sternal markings. ♂ (Accra). *E*, lateral view of extruded genitalia; *H*, posterior dorsal view of the same. ♂ (Zanzibar). *F*, genitalia (retracted).

♂. The sexes are extremely alike. In the ♂ the head is a little longer and the sides behind the antennae straighter than in the female. The antennae are relatively larger and stouter than in the female, and with practically the same absolute measurements; the second joint is a trifle longer, and distinctly contracted basally, so that the profile is subtriangular; the fourth joint is longer than broad. The postero-lateral spine is longer than in the female, three-fifths the width of the head. Chaetotaxy of head, legs and thorax as in the female.

Abdomen. Bristles of pleurites as in the female; the long pairs on 7 and 8 slightly more developed. Tergites 1 and 2 bear three rows of bristles in all (2, 4, 8); tergites 3–8 have a single row (7–9); while on tergite 9 there are four minute bristles. Sternites 2–6 inclusive bear two rows of bristles (7, 6) and there

is a single row (6) on the seventh sternite, and two bristles on the eighth; the ninth has two moderately long terminal bristles, with two minute between and a pair (minute) outside each of the longer bristles.

In the distribution of the chaetotaxy three regions are recognised. Except posteriorly, there is on each segment a distinct gap between the bristles of the tergites and those of the pleurae, or between the latter and those of the sternites.

Genitalia. The paramer is a short, rather broad apically rounded lobe which in the exserted state (Fig. 2, *E* and *H*) (when the parts fold back over the abdomen) is outermost (*i.e.* posterior or ventral) having thus reversed the position occupied at rest (Fig. 2, *F*). What I believe to be the upper endomers are two narrow finely pointed clear chitinised processes which seem straight in the retracted condition, viewed from above, but when exserted each is curved and outspread so that together they have a lyriform facies. The triangular pseudopennis (the lower endomer) appears to emit the sac, which is nowhere distinctly chitinised, from its upper surface, near the base.

	Length	Breadth
Head	0·34	0·17
Thorax	0·18	0·28
Abdomen	0·88	0·54

Total length 1·35 mm.

Type. ♂ in the collection of the British Museum.

One of a series (presented by the Imperial Bureau of Entomology), 2 ♂♂, 7 ♀♀ and 10 immature, from *Cricetomys gambianus*, ACCRA: 6. ii. 1915; No. 519 (Dr J. W. Scott Macfie); and 1 immature with same data, 3. xii. 15.

In the general collection of the British Museum there are also 2 males (1 damaged) from *Cricetomys*, Zanzibar.

In *P. calva* some of the generic characters are feebly marked. The species, though very distinct, occupies an intermediate position in our present classification. The larvae are *Linognathus*-like and only with the last moult do the characters of *Polyplax* appear. Superficially, at least, its nearest allies are in *Linognathoides*, Cummings (1914), but it is possibly best to restrict that name to the squirrel-infesting lice.

SCLEROSTOME PARASITES OF THE HORSE IN ENGLAND[1].

II. NEW SPECIES OF THE GENUS *CYLICHNOSTOMUM*

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(With 5 Text-figures.)

CONTENTS.

Introduction.

In a recent number of *Parasitology* (1916) an account was given of the species of *Triodontophorus* and *Oesophagodontus* found in the alimentary tract of horses in the neighbourhood of Birmingham, more particularly in the Redditch district of Worcestershire. In company with the worms of these two genera were several species of *Cylichnostomum*, occurring in some of the hosts in considerable numbers both in the colon and caecum.

The majority of these parasites could be referred to species described in Egypt by Looss (1900, 1901); three, however, proved to be new and form the main subject of the present paper.

Leiper (1910) has recorded the occurrence of a few species of *Cylichnostomum* in horses from the London district, but apart from this

[1] Owing to the author's absence abroad the proofs of this paper have not been submitted to him for correction.—Ed.

brief communication no attempt has been made to determine the British representatives of the genus since Mehlis' old species *C. tetracanthum* was broken up by Looss (1900, 1901).

Altogether eight species were obtained by me in the Midlands; in addition to the three new forms which I have named *C. euproctus*, *C. insigne* and *C. goldi*, the following were also observed:

C. poculatum Looss.
C. calicatum Looss.
C. nassatum Looss.
C. coronatum Looss.
C. bicoronatum Looss.

It is interesting to note that *Cylichnostomum tetracanthum* (Mehlis) Looss is not in this list, neither was it observed by Leiper. As Looss was unable to obtain Mehlis' original paper (1831) he decided to choose as type species that form which occurred most commonly in Egypt; if this species proves to be absent from European countries his choice will turn out to have been an unfortunate one.

There is, however, nothing to gain from Mehlis' account of his *Strongylus tetracanthus* which is very scanty; I have looked up his paper in *Oken's Isis* (1831) but the few lines devoted to this worm would apply equally well to many other species of *Cylichnostomum*, the only definite and useful information refers to the size of the worms, which is given as 6–7 lines, *i.e.* about 15–17 mm., this is certainly larger than Looss' *C. tetracanthum* sensu stricto.

The diagnoses of the new species observed are given below:

Genus Cylichnostomum Looss 1901.
Cyathostomum Molin 1861.
Cylicostomum Railliet et Henry 1902.

Cylichnostomum euproctus sp. n.

Specific diagnosis. *Cylichnostomum*[1]: Head marked off from the body by a slight constriction (Text-fig. 1 A, B), its diameter 110–130 μ. The mouth-collar is comparatively high (33–46 μ) and somewhat flattened towards its lateral margins so as to appear nearly hemispherical when seen in lateral view (Text-fig. 1 A). The lateral head papillae are not prominent, the submedian head papillae are very short and spherical in shape.

[1] For a definition of the genus *Cylichnostomum* cf. Looss (1901).

The external leaf-crown is composed of about 40 narrow and pointed leaves; the elements of the internal leaf-crown are more conspicuous, the latter consisting of 30–34 long and somewhat refractive leaves, recalling those of *C. bicoronatum* Looss (Text-fig. 1 A, B).

The mouth-capsule has a breadth of 75–100 μ and a height of 28–40 μ, its walls are thickest near the middle, thinning down anteriorly and posteriorly, the shape as seen in optical section varies a little in different

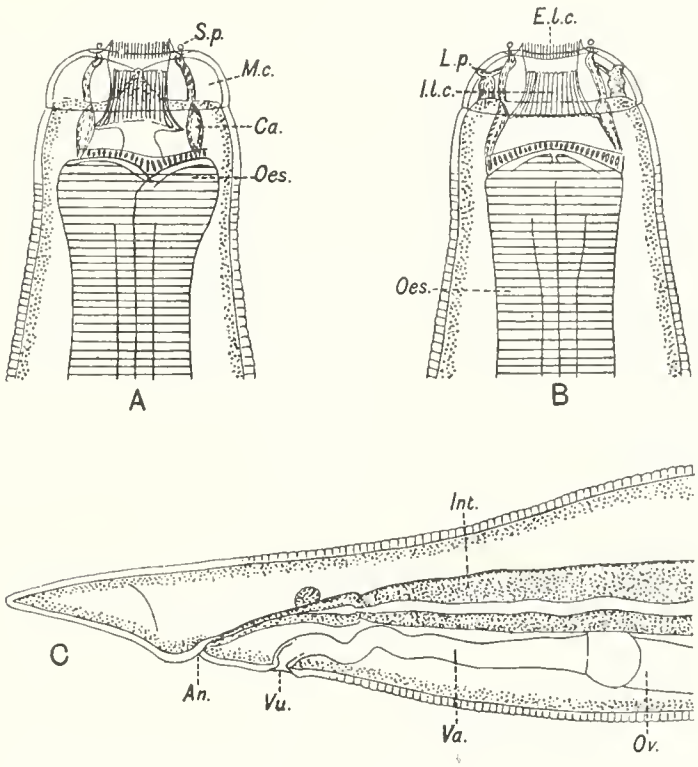

Fig. 1. *Cylichnostomum euproctus* sp. n. A. Lateral view of anterior extremity, × 140. B. Dorsal view of anterior extremity of another specimen, × 140. C. Posterior extremity of body of female seen from right side, × 72.

specimens; extremes are shown in Text-fig. 1 A, B. There is no dorsal gutter, the oesophageal gland opening at the base of the capsule.

The oesophagus is 360–420 μ long and approximately flask-shaped, it is widest near its posterior extremity where it attains a breadth of 110–138 μ.

The excretory pore and the cervical papillae are situated far back, only a short distance in front of the posterior end of the oesophagus and 360–500 μ from the anterior extremity of the body (Text-fig. 2 A).

Female: 6–8 mm. in length. The body attains a maximum thickness of 350–450 μ near the middle.

The vulva is 300–400 μ from the posterior extremity of the body. The "tail" region appears pointed and tapers gradually behind the vulva, at the level of which the breadth of the body measures 140–200 μ. The axis of the "tail" is in a straight line with that of the body (Text-fig. 1 C) or only very slightly bent towards the dorsal surface. The anus is 190–250 μ from the posterior extremity.

The vagina is very long (400–460 μ).

The eggs measure 80–100 μ in length and 50–60 μ in breadth.

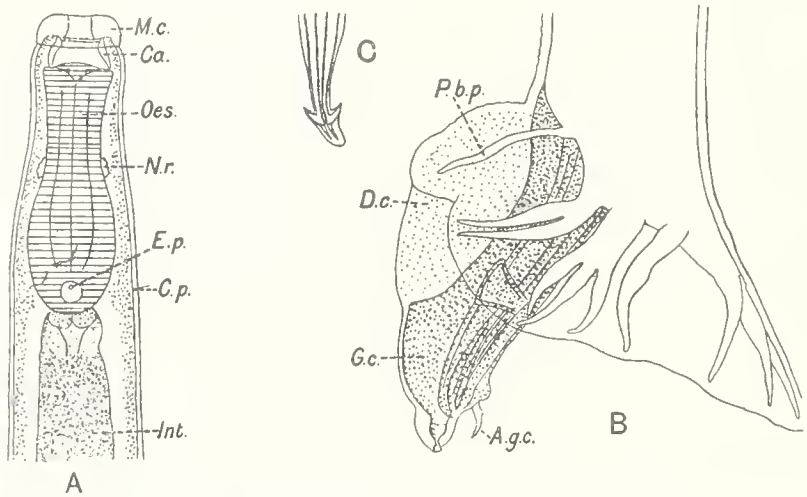

Fig. 2. *Cylichnostomum euproctus* sp. n. A. Anterior end of the body in ventral view, × 72. B Lateral view of male bursa and genital cone, × 72. C. Terminations of the spicules, × 375.

Male: 6–7·2 mm. in length, maximum thickness 350–390 μ. The bursa has a dorsal lobe of moderate length (Text-fig. 2 B); the most striking character of the species is found in the genital cone which has a relatively enormous length (310–400 μ), the dermal collar is greatly developed on its ventral face and the prebursal papillae are thus very elongated (160–220 μ). The appendages of the genital cone are long, narrow and "cirrus" shaped (Text-fig. 2 B).

The spicules are rather strong, their terminations are shown in Text-fig. 2 C.

Cylichnostomum euproctus occupies a somewhat isolated position and is difficult to group with any other species of the genus. In some respects, *e.g.* in the configuration of the head, it most nearly approaches *C. bicoronatum*.

This species was found on several occasions in considerable numbers both in the colon and caecum.

Cylichnostomum insigne sp. n.

Specific diagnosis. *Cylichnostomum*: Head not separated from the body, 190–250 μ in breadth. The mouth-collar is comparatively narrow and separated from the rest of the cuticle by a marked constriction.

The lateral head-papillae are prominent and project from the surface of the mouth-collar to form "horn"-like processes, less developed, however, than those of *C. auriculatum* Looss. The submedian head-papillae are short and leaf-shaped (Text-fig. 3 A).

The external leaf-crown consists of about 36 large pointed leaves, the leaves of the internal crown are however very inconspicuous, the latter appearing as a finely striated zone immediately in front of the mouth-capsule (Text-fig. 3 A).

The mouth-capsule is large having a depth of 46–66 μ and a maximum breadth of 130–200 μ. The walls of the capsule are considerably increased posteriorly so as to form a hoop-like thickening similar to that found in *C. elongatum* Looss and *C. auriculatum* Looss.

There is no dorsal gutter and the oesophageal funnel is scarcely developed (Text-fig. 3 A).

The oesophagus is long, 700–900 μ, rather narrow anteriorly, it broadens out behind the level of the nerve-ring and attains a maximum breadth of 200–330 μ.

The excretory pore is situated at approximately the level of the junction of oesophagus and intestine, its position is therefore a little further forwards in the body than that of *C. auriculatum* (Text-fig. 3 B).

The cervical papillae occur just in front of the excretory pore, 900–950 μ from the anterior extremity.

Female: 13·5–15 mm. in length, the maximum breadth near the middle of the body is 740–850 μ.

The vulva is relatively close to the posterior extremity (300–430 μ); at this level the body has a breadth of about 300 μ, it tapers gradually behind this point to the level of the anus, 160–250 μ from the posterior end. Behind the anus the "tail" narrows suddenly (Text-fig. 3 C) and forms a little pointed tip usually bent in a dorsal direction. The subcuticular tissue in the neighbourhood of the vulva is usually thickened in places so as to form irregular swellings, sometimes in the shape of

almost complete transverse rings, similar to the "clitellum"-like thickenings described by Looss in other species.

The vagina is very long, measuring 1·4–1·6 mm.

The eggs measure 75–86 μ in length with a breadth of 45–50 μ.

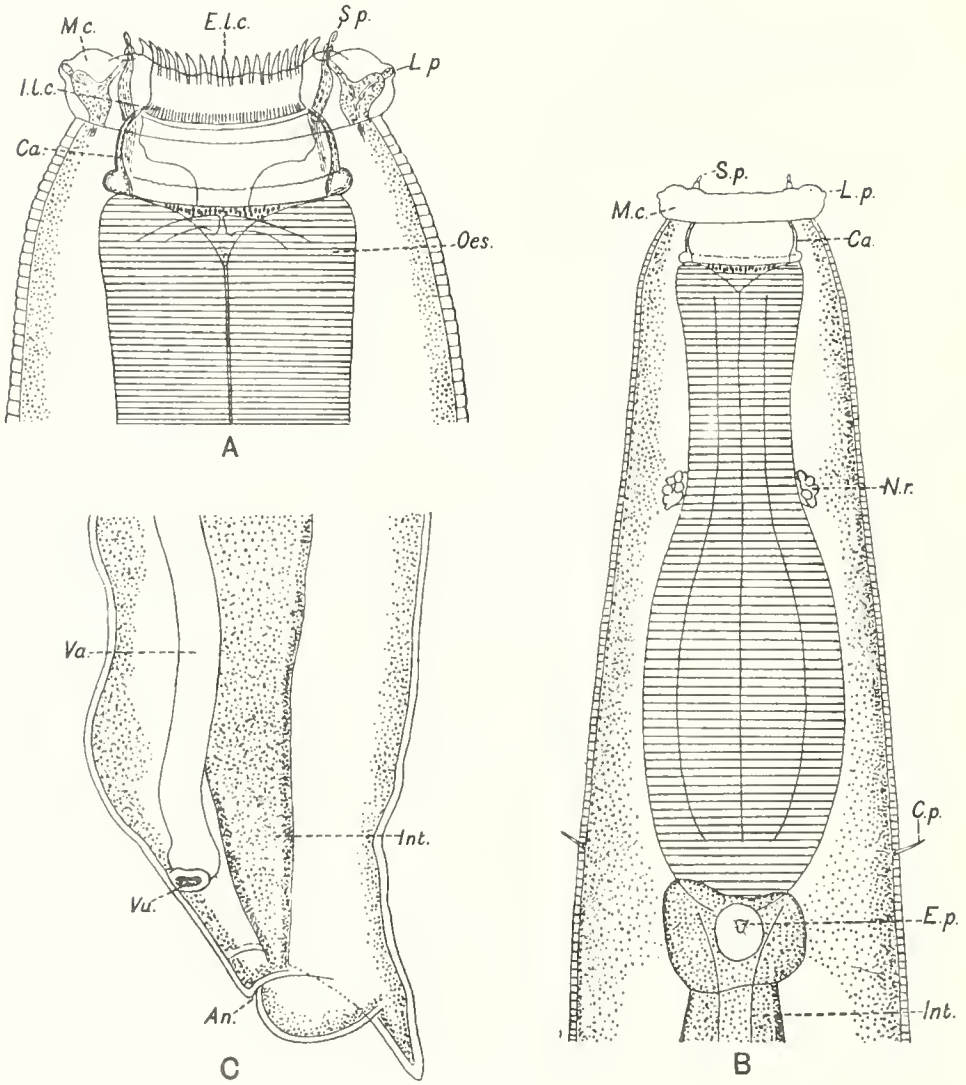

Fig. 3. *Cylichnostomum insigne* sp. n. A. Anterior extremity in dorsal view, × 140. B. Anterior end of the body in ventral view, × 72. C. Posterior extremity of body of female seen from left side, × 72.

Male: 11–12 mm. in length, the body attains a maximum thickness of 620–690 μ.

The bursa (Text-fig. 4 A) has a long, broad median lobe.

The dermal collar of the genital cone is well developed, especially on the ventral surface. The appendages of the genital cone (Text-fig. 4 B, *A.g.c.*) are broad and fused together in the middle line, each bears a little papilla-like process.

Cylichnostomum insigne is undoubtedly closely allied to *C. auriculatum* Looss, the species is however a little smaller in size and differs from the latter in a number of characters; thus the horn-like processes formed

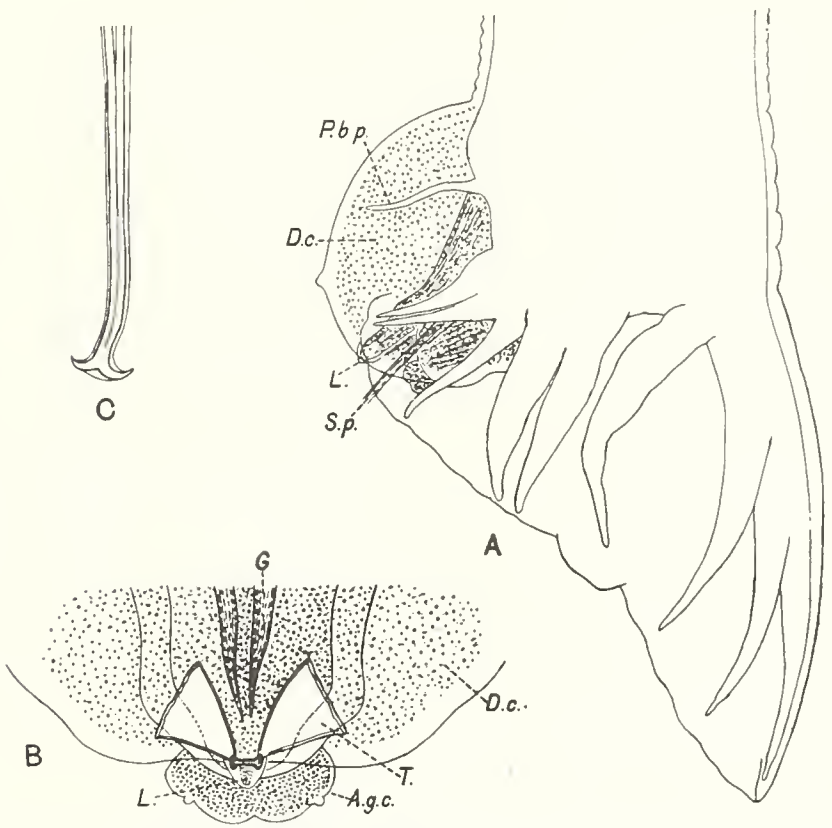

Fig. 4. *Cylichnostomum insigne* sp. n. A. Lateral view of male bursa, × 72. B. Ventral view of the genital cone to show the appendages, × 140. C. Terminations of the spicules, × 375.

by the lateral head-papillae are less developed than in *C. auriculatum* and the cervical papillae and excretory pore are situated more anteriorly than in that species, although considerably further back than in the majority of the species of the genus. In some respects *C. insigne* is intermediate between *C. auriculatum* and *C. elongatum* Looss.

The species was found on one occasion only, in small numbers, at Beoley, near Redditch, Worcestershire.

Cylichnostomum goldi sp. n.

Specific diagnosis. *Cylichnostomum*: Body small and rather delicate. Head continuous with the body, 80–100 μ in breadth. The mouth-collar is rather high and distinctly separated from the rest of the skin. The lateral head-papillae are very inconspicuous, the submedian small and conical in shape (Text-fig. 5 A).

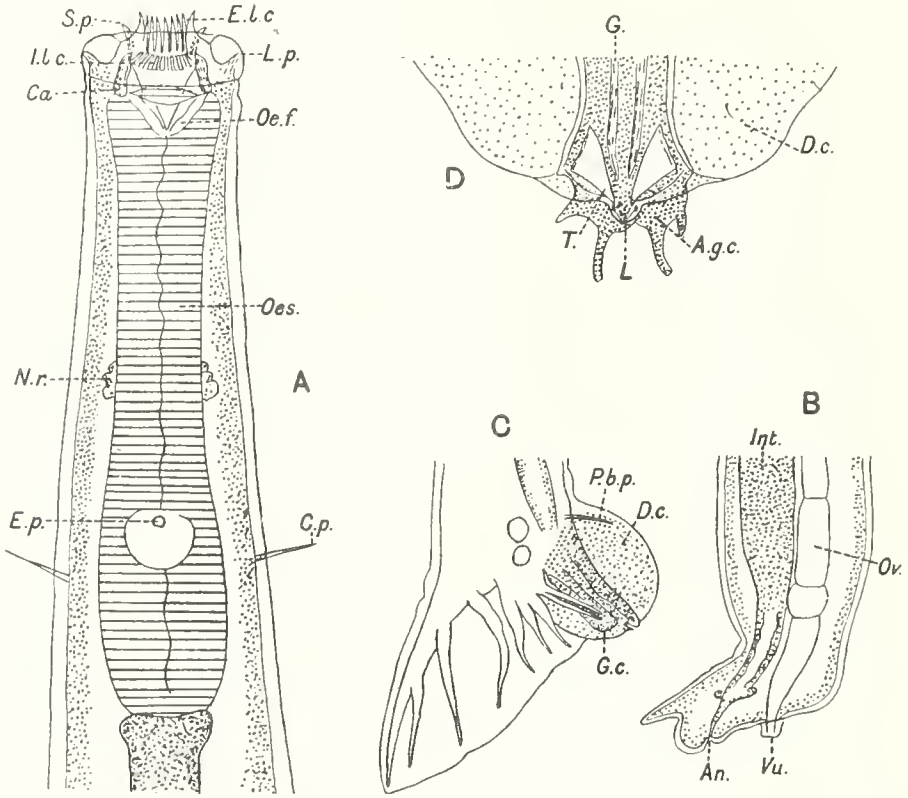

Fig. 5. *Cylichnostomum goldi* sp. n. A. Anterior extremity of the body in ventral view, ×215. B. Lateral view of posterior extremity of female, ×72. C. Lateral view of male bursa, ×72. D. Ventral view of the genital cone to show the appendages, ×215.

In general shape and arrangement the mouth-capsule and the leaf-crowns resemble those of *C. alveatum* Looss and *C. catinatum*; the elements of the external leaf-crown are large and leaf-like and number about 20; the internal leaf-crown is composed of 30–32 shorter leaves.

The mouth-capsule has its walls thickened in much the same manner as in *C. catinatum*, it measures 45–60 μ in breadth and about 20 μ in height.

The oesophageal funnel is very well developed, there is no dorsal gutter (Text-fig. 5 A).

The oesophagus is long (300–350 μ) and somewhat slender, being not markedly swollen behind the level of the nerve-ring, its maximum breadth is 70–75 μ.

The excretory pore is situated rather far back on the body, at a level about half-way between the nerve-ring and the posterior extremity of the oesophagus, *i.e.* 250–300 μ from the anterior end of the body (Text-fig. 5 A).

The cervical papillae are situated close behind the excretory pore (Text-fig. 5 A), 270–300 μ from the anterior extremity.

Female: 6–6·7 mm. in length, maximum breadth 280–300 μ. The whole tail-region (Text-fig. 5 B) is bent dorsally and takes up a position almost at right angles to the main axis of the body. The vulva is situated about 200 μ from the posterior extremity.

The anus is 100–110 μ from the posterior end of the body, behind this level the tail is suddenly narrowed and terminates in a slender point.

The vagina is short (Text-fig. 5 B), the eggs have an average length of 100 μ by an average breadth of 50 μ.

Male: 5·2–6 mm. in length, with a maximum body breadth of 230–280 μ The bursa (Text-fig. 5 C) has a short dorsal (median) lobe which when spread out appears approximately semicircular. The dermal collar is well developed both on the anterior and posterior surfaces of the genital cone. The appendages of the genital cone are very characteristic (Text-fig. 5 D, *A.g.c.*) having the form of very thin delicate plates, each provided with two slender, finger-shaped processes.

Cylichnostomum goldi was found on several occasions both in the colon and caecum, always in small numbers. It obviously belongs to the group of species which includes *C. alveatum* and *C. catinatum*.

I have named the species after Mr J. A. Gold, M.R.C.V.S., of Redditch, to whom I wish again to express my thanks for his kindness in supplying me with material from infected horses in his district.

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EXPLANATION OF LETTERING.

A.g.c. Appendage of the genital cone.
An. Anus.
Ca. Wall of mouth-capsule.
C.p. Cervical papilla.
D.c. Dermal collar of the genital cone.
E.l.c. External leaf-crown.
E.p. Excretory pore.
G. Guiding piece (gubernaculum).
G.c. Genital cone.
I.l.c. Internal leaf-crown.
Int. Intestine.
L. Ventral lip of the genital cone.
L.p. Lateral head-papilla.
M.c. Mouth collar.
N.r. Nerve-ring.
Oes. Oesophagus.
Ov. Ovejector.
P.b.p. Prebursal papilla.
Sp. Spicule.
S.p. Submedian head-papilla.
T. Triangular plate of the genital cone.
Va. Vagina.
Vu. Vulva.

ON THE DEVELOPMENT OF *ASCARIS LUMBRICOIDES* LIN. AND *ASCARIS SUILLA* DUJ. IN THE RAT AND MOUSE.

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(With Plate I and 9 Text-figures.)

In publishing the following paper the author wishes to express his thanks to several gentlemen who have kindly assisted him in the investigation described, to Dr Johnson, Principal Civil Medical Officer of Hong Kong, and to Dr Macfarlane, Government Bacteriologist, Hong Kong, for their kindness in permitting him to use the Bacteriological Institute of the Colony; to Dr Macfarlane for much assistance during the year occupied by the research, and to Mr A. Gibson, Colonial Veterinary Surgeon, for great help in the supply of material.

The development of *Ascaris lumbricoides* is at present almost universally considered to be direct. It is well known that the eggs passed in the faeces of man undergo development in the outer world up to the formation of a motile vermiform embryo. The egg containing this embryo may readily reach the alimentary canal of man and it is supposed that it there hatches and that the larva having escaped develops in this site into the adult. This hypothesis is based on the work of Davaine (1858, 1863), Grassi (1887–8), Calandruccio (1886), Lutz (1887–8), Epstein (1892), Jammes and Martin (1906–8), Martin and Wharton (1915). I have not at present access to the original papers of these authors with the exception of those of Jammes and Martin and of Wharton. Summaries sufficient for the present purpose are, however, available in the text books of Leuckart (1867) as regards Davaine, of Railliet (1895), Manson (1908), Allbutt (1909), and Castellani and Chalmers (1913) as regards the other writers.

Davaine administered ripe eggs to rats and found that after twelve hours free, living larvae were to be found in the lower part of the small intestine. He also introduced ripe and unripe eggs in glass capsules closed with linen into the alimentary canal of the dog and found that after the lapse of a certain period the ripe eggs had disappeared, whereas the unripe eggs remained. He concluded that hatching and development occurred in the alimentary canal of the definitive host. Grassi administered ripe eggs to himself and two months later found eggs in his stools. Calandruccio successfully infected a child of ten which had previously suffered from worms but had been relieved of these parasites by anthelmintics. Lutz fed an adult on ripe eggs and found evidence of the subsequent appearance of adult worms. Epstein's work is unfortunately not available even in summary, but from the context of the references it is clear that he successfully infected man.

Jammes and Martin allowed ripe eggs to hatch in artificial and natural solutions and found that hatching took place readily and *en masse* in 0·8% salt solution (which they consider to be an alkaline solution) at 37°–40° C. Martin (1913, cited by Wharton) finds that the embryos of the ascarids from the calf, pig, horse and dog, hatch best in alkaline solutions, and that when developed eggs are introduced into the alimentary canal of an animal they pass through the stomach unaffected and only hatch after they have been subjected to the action of the alkaline juices of the intestine. Wharton states that direct infection can take place, but that the embryos must be "completely developed." He does not give the period necessary to secure this complete development.

In spite of the general acceptance of the hypothesis that infection takes place by the direct method there is a considerable bulk of evidence against it.

Davaine administered three to four hundred ripe eggs to an ox, an animal which is stated to harbour *Ascaris lumbricoides*, and found that after four months no worms were present in the intestine.

Leuckart (1867) fed a rabbit on ripe eggs and found no worms after ten days; a dog was also treated and was equally unresponsive after fourteen days. This very experienced helminthologist also fed a pig for three weeks on several thousand ripe eggs and did not find any worms on section. An experiment on man was arranged for in 1857 but it is not clear that it was carried out. He also administered to a horse the eggs of *Ascaris megalocephala*, to a dog those of *A. marginata*, to a cat those of *A. mystax*, with invariably negative results. Leuckart therefore maintained that the life-history of *A. lumbricoides*

would be found to be completed by an alternation of hosts. He supported this opinion by the facts known with regard to (1) *A. acus* which is found as an encysted larva in *Leuciscus alburnus* and in the adult form in the pike, and (2) a larval *Ascaris* found encysted in the muscles of the mole which when administered to the buzzard (*Falco buteo*) continues to develop although it does not yet become adult.

Leuckart considered that Davaine's rat experiment, if correctly described, did not point to the direct development of the worm but to the fact that the rat was an intermediate host. He pointed out the ease with which the larvae liberated in the faeces of the rat would be conveyed to the intestine of the definitive host,—man. He attempted to confirm the experiment but employed a mouse in place of a rat, and found that the eggs were passed unaltered in the faeces of the mouse. He therefore abandoned this line of research. He attempted to find the intermediate host among invertebrate animals experimenting with a number of insects, snails and earth-worms but without success.

Von Linstow (1886) suggested that *Julus guttulatus* might be the intermediate host.

The Author's Observations.

Ascariasis both in man and the pig is of extraordinary frequency in the colony of Hong Kong and in South China generally, the author therefore while stationed in this colony had an unusual opportunity of studying the subject. He commenced experiments in the spring of 1915.

(1) Two young pigs were obtained aged two months. The faeces of both were examined and found to be free from *Ascaris* eggs.

Pig *A* was fed throughout the course of the experiment on tinned milk and rice flour. Large quantities of ripe eggs from the *Ascaris* of the pig were administered to it on thirteen occasions between the 20. ix. and 6. x., 1915. The age of the eggs employed varied from 26 to 64 days and they invariably contained well developed and motile embryos. They had been incubated at 25°–30° C. in a damp atmosphere. The eggs were administered under varying conditions, after food and after twelve hours' starvation, with and without the addition of bicarbonate of soda. The total number of eggs used must have greatly exceeded several thousand. This pig was killed 15. xii., 1915. One small *Ascaris* only was found in its intestine.

Pig *B* was fed between ix. and xii. 1915 in the same manner as pig *A*, on tinned milk and rice flour; from i. 1916 onward he was fed on boiled rice and vegetables. Between 27. ix. and 2. xii. 1915 large quantities of ripe eggs from the *Ascaris* of man were administered to him on nine occasions. The age of the eggs varied from 22 to 106 days. They also had been kept during the colder months in an incubator between 25° and 30° C. in a damp atmosphere. The faeces of the pig were repeatedly examined for *Ascaris* eggs but none were found. Between 5. i. and 27. ii. 1915, large quantities of eggs from the *Ascaris* of the pig were administered, the age of the eggs varying between 17 and 73 days. The faeces of this animal were again examined repeatedly but up to 17. iv. no eggs of *Ascaris* were found.

These two series of experiments thus strongly confirmed the negative finding of Leuckart's experiment with the pig.

(2) On 6. iv. 1916 the writer took up Davaine's experiment with the rat. Four specimens of *Mus decumanus* (albino) had been obtained. Their faeces had been repeatedly examined and no eggs of nematodes had been found. At 2 p.m. on 6. iv. ripe eggs of *Ascaris lumbricoides* from man were administered to all four rats. The faeces passed between 8 p.m. on the 6th and midday on the 7th were found to contain free larvae of *A. lumbricoides*. These larvae moved in a languid manner in normal salt solution. Eggs of the *Ascaris* of the pig were administered to all four rats on 7 and 9. iv. and to rats *A*, *B* and *D* on 10. iv. Eggs of the *Ascaris* of man were again given to rat *C* on 10. iv. The faeces continued to contain free *Ascaris* larvae. Specimens of the faeces were preserved in an incubator at 25°–30° C. Living larvae were found in these specimens after the lapse of three days. The experiment of Davaine was therefore fully confirmed.

On 12. iv. a further development of the experiment took place. Rat *C* died. The writer was prevented from examining this rat or observing the remaining three until 15. iv. Rat *C* was preserved during the interval in an ice chest. On 15. iv. it was examined. A small quantity of blood had escaped from its nostrils. No nematodes, larval or adult, were found in the stomach or intestines. The lungs were found to be congested. Portions were removed and teased out in normal salt solution. Numerous nematode larvae in active movement escaped from the tissue. The liver was also examined and a small number of larvae found. No larvae were found in the spleen or kidneys.

The rats *A*, *B* and *D* were on this day obviously suffering from pneumonia. A small quantity of blood was issuing from the nostrils

of *B* and *D*, and all three were breathing in a rapid and exaggerated manner.

On 16. iv. rat *D* was killed. The same nematode larvae were found abundant in the lungs. No larvae were found in the trachea, liver, heart, spleen, kidneys, stomach, intestine or in the masseter and lumbar muscles.

Rat *A* had apparently recovered from its illness on 17. iv., rat *B* on 18. iv.

The organs of *C* and *D* were examined by serial sections. In the lungs the greater part of the air vesicles were found to be filled with red blood corpuscles. Larvae were found in the air vesicles (Pl. I, fig. 4) and in the bronchi of *D* (Pl. I, fig. 8).

No larvae were found in the other organs examined, viz. liver, kidneys and spleen in the case of *D*, kidneys and spleen in the case of *C*.

As a control another specimen of *Mus decumanus* (albino), *E*, obtained from the same source as *A*, *B*, *C* and *D*, and five specimens of the wild *M. decumanus* and two of *M. rattus* obtained from the town of Victoria were examined. No larvae were found in their lungs.

The rat *B* was killed on 22. iv. The lungs, trachea, nasal cavities, liver, heart, spleen, stomach and intestines were examined. No larvae or other worms were found. The lungs appeared slightly fibrosed but otherwise normal. The rat had therefore freed itself from the parasites 16 days after the date of the first infection and 12 days after the date of the last infection.

(3) Further experiments were made to confirm the result obtained. On 22. iv. two white rats *F* and *G* were given large quantities of mature eggs. Rat *F* was treated with the eggs of an *Ascaris* of the pig which were 80 days old. On 25. iv. the treatment was repeated. On the 27th the rat was obviously ill and breathing at the rate of 134 per minute. It continued seriously ill until 4. v. when it was killed. The lungs were almost solid from pneumonia and were markedly soft and gritty when teased. The following organs were carefully searched with a dissecting microscope—nasal cavities, surface of the tongue. trachea, oesophagus, lungs, kidney, spleen and the entire alimentary tract. No nematodes were found with the exception of one dead larva (probably an *Ascaris*, vide infra) in the stomach, and one dead and partially digested larva of the same character in the rectum.

This rat had therefore become almost completely free of infection in twelve days from the date of first infection and nine days from the last.

(4) Following Davaine's experiment with the dog the writer administered very large doses of ripe eggs of both the *Ascaris* from man and of that from the pig to a puppy four months old. It did not appear to be in any way affected by the dose.

(5) Rat *G* was fed with eggs of the human *Ascaris* 54 days old. The doses were repeated on the 24th and 25th. On the 26th it was extremely ill and breathing at the rate of 160 per minute. On the 27th it died. *Ascaris* larvae were found abundant in the lungs and liver.

(6) A piebald mouse (mouse *A*) said to be one year old was treated with the same culture as rat *G*, on the 24th, 25th and 26th of April. On the 28th it was seriously ill, with respirations at the rate of 120 per minute. It died on that day. Lungs and liver were richly infected with larvae.

It is interesting to compare the last experiment with that of Leuckart's with the mouse. Leuckart asserted that the eggs were passed in the faeces unaltered. The present writer found in addition to a few unaltered eggs a small number of free but dead larvae. It is probable that Leuckart did not give sufficiently large doses of eggs to cause the death of the animal and thus failed to observe the larval stages of the parasite.

In the cases of rat *C* and mouse *A*, sections proved that the larvae were situated in the air vesicles of the lungs and in the blood capillaries of the liver close to the interlobular veins (Text-fig. 9).

(7) A second mouse (mouse *B*) was taken for experiment on 5. v., when a small dose of ripe eggs of *Ascaris suilla* was administered to it. On 12. v., that is the 7th day after infection, it was observed to be ill. It was kept at that time in a box which was furnished with a darkened upper ledge resembling a mouse's hole. The mouse was observed to leave this hole and sit in the open box. On 13. v., the 8th day after infection, it was killed. Larvae measuring roughly from 1 to 1·5 mm. in length were found in the roots of the lungs—doubtless in the bronchi. One larva measuring 1 mm. and one of 1·5 mm. were found in the trachea. No worms were found in the nasal cavities, liver, or alimentary canal.

(8) Having traced the infection to the air vesicles, bronchi and trachea of the rat and mouse it became necessary to ascertain whether the larvae in these situations were capable of further development in the definitive host. Portions of the lungs of rat *D* were administered on 16. iv. to the pig *B* which had been used in the experiments on direct infection described above. This animal was killed on 30. iv.

and stomach and intestines, heart, lungs and liver were examined. No ascarids were found in any of these organs.

Several factors may have been responsible for this failure: (1) The larvae may require to undergo further development either in the outer world or in a second intermediate host. (2) The larvae in the lung of rat *D* may have originated only from the first dose of eggs administered to it and may therefore have belonged to the *Ascaris* of man. It is of course a point in debate whether the human ascarid (*A. lumbricoides* Lin.) is or is not specifically identical with that of the pig (*A. suilla* Dujardin). Infection experiments only will be able to decide this point. (3) The pig *B* may have been rendered immune to *Ascaris* infection by the large doses of eggs administered to it previously. The eosinophil index of the blood was observed to rise after several of these administrations.

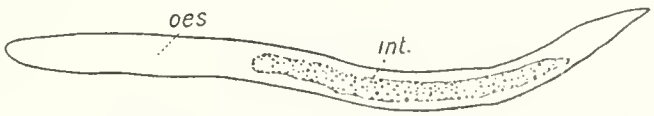

Fig. 1. Larva of *Ascaris suilla* in the faeces of the rat, 48 hours after evacuation. In salt solution. ×340.

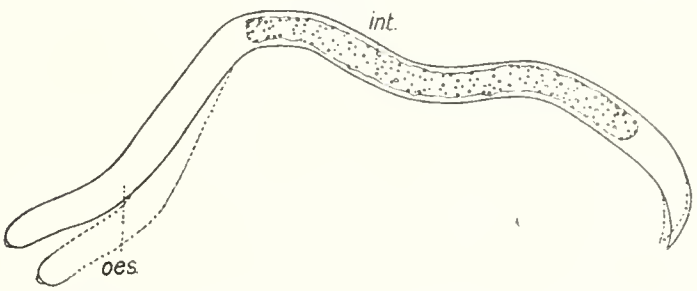

Fig. 2. Larva of *Ascaris suilla* in the faeces of the rat, 30–40 hours after evacuation. The second outline shows the range of movement observed. ×340.

Description of Larvae found in the Rat and Mouse.

Absolute and relative measurements of larvae at different stages of development.

To obtain reliable absolute measurements specimens have been sketched and measured either in 0·8 % salt solution or in weak corrosive sublimate solution.

Text-figs. 1 and 2 represent larvae passed in the faeces of the rat; Fig. 1 forty-eight hours and Fig. 2 thirty to forty hours after evacuation.

The larvae are 0·22 mm. and 0·28 mm. in length, oesophagus length 0·084 mm. and 0·11 mm., the proportion of length of oesophagus/total length 1/2·6 and 1/2·5.

Text-fig. 3 is from a larva in the lung of the rat five days after the first infection and two days after the last infection. The larva measures 0·5 mm. in length, head to nerve ring 0·067 mm., length of oesophagus 0·135 mm., length of oesophagus/total length 1/3·7.

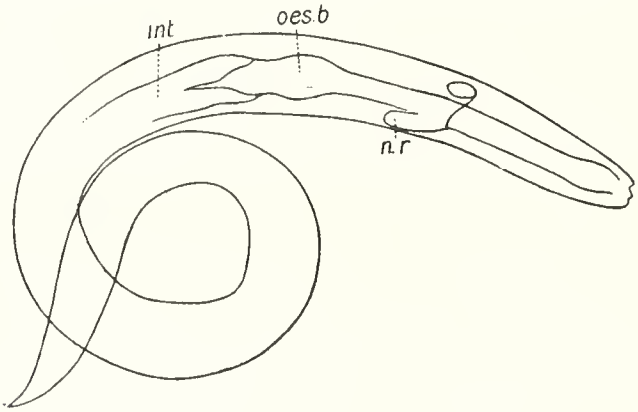

Fig. 3. Larva from the lung of rat *G*, in weak corrosive sublimate solution. ×340.

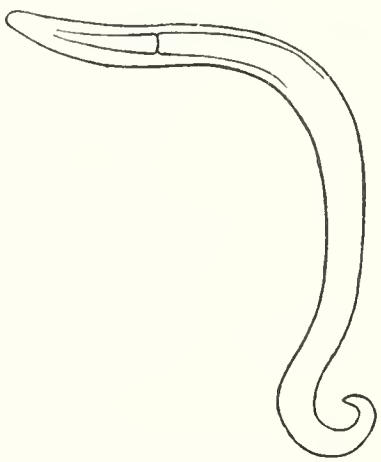

Fig. 4. Larva from the lung of rat *C*, in weak corrosive sublimate solution. ×102.

The specimens from which Text-figures 3, 4 and 5 are drawn were killed and mounted in weak corrosive sublimate solution.

Text-fig. 4 gives the outline of a larva from the lung of the rat six days after the first and two days after the last infection. Total length 0·84 mm., length of oesophagus 0·17 mm., length of oesophagus/total length 1/4·9.

Text-fig. 5. A larva from the lung of the rat ten days after the first infection and six days after the last. Total length 1·4 mm., length of oesophagus 0·227 mm., length of oesophagus/total length 1/6·1. This was the largest larva observed in the lung.

(1) Larva from the liver of the mouse four days after the first and two days after the last infection. (Pl. I, fig. 2.)

Total length 0·143 mm., maximum breadth 0·01 mm., head to nerve ring 0·018 mm., length of oesophagus 0·051 mm., length of oesophagus/total length 1/2·8, anus to tail 0·017 mm.

Three lips. In the oesophageal region, epidermal cells form a complete sheath for the oesophagus representing the future longitudinal lines, nerve collar and excretory system.

The ventral gland (rudiment of the excretory system) cannot be recognised but it is doubtless one of the larger cells of the ventral line posterior to the nerve ring. The body of the oesophagus is represented by two groups of nuclei (*oes.* and

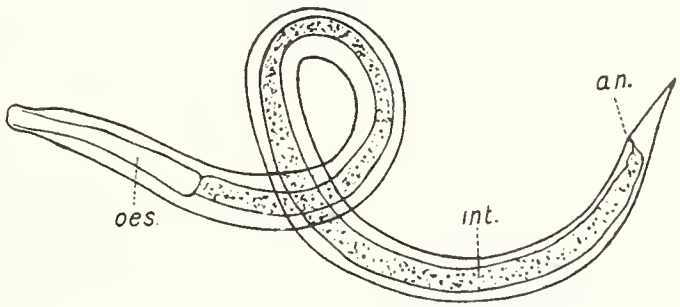

Fig. 5. Larva from the lung of rat *D*, in weak corrosive sublimate solution. × 102.

oes. b.). The latter have the appearance of a rudimentary oesophageal bulb. In the intestinal region, all four longitudinal lines are distinguishable (*V.L.*, *D.L.*, *R.L.L.*, *L.L.L.*). The ventral line is by far the most developed. The connection of this line with the large cells of the ventral portion of the oesophageal collar is quite clear. Pigment granules mark the walls of the intestine. The caudal mass is perforated by a rudimentary anal canal.

(2) Larva from the lung of the mouse four days after the first and two days after the last infection. (Mounted in Canada balsam.)

Total length 0·161 mm., maximum breadth 0·011 mm., head to nerve ring 0·019 mm., length of oesophagus 0·048 mm., length of oesophagus/total length 1/3·35, anus to tail 0·014 mm.

Lips are not distinguishable in the preparation. Large nuclei surround the oesophagus, interrupted by the nerve ring. Very few intestinal pigment granules. Posterior to the oesophagus three classes of nuclei can be distinguished: (1) those of the ventral line, strongly staining and small; (2) large faintly staining nuclei probably belonging to the lateral line; (3) smaller faint nuclei probably

the rudiments of the muscle fields. The caudal group of nuclei which is so prominent a feature in the embryo is still marked. It is perforated by the rudiment of the anal canal leading to the rudiment of the anus. No trace of ventral gland.

(3) Larva from the lung of the same mouse. (Mounted in Canada balsam.)

Total length 0·176 mm., maximum breadth 0·016 mm., head to nerve ring 0·026 mm., length of oesophagus 0·052 mm., length of oesophagus/total length 1/3·37.

The ventral line is prominent. Rudimentary anal canal and anus distinguishable. A large granular cell in the posterior oesophageal region may represent the ventral gland.

(4) Larva from the lung of the rat five days after the first infection and two days after the last. (Mounted in Canada balsam.) (Pl. I, fig. 3.)

Total length 0·26 mm., head to nerve ring 0·038 mm., length of the oesophagus 0·076 mm., length of oesophagus/total length 1/3·4.

The nerve ring is distinct; the ventral gland is not visible; the ventral line is still very strongly developed; the anus is marked by a depression.

(5) Larva from the lung of the rat six days after the first infection and two days after the last. (Mounted in Canada balsam.)

Total length 0·33 mm., head to nerve ring 0·04 mm., head to end of oesophagus 0·095 mm., length of oesophagus/total length 1/3·47.

The three lips are clearly marked, nerve ring less so than in the earlier stages, ventral gland more developed and ventral line relatively less so.

In one specimen of about this stage killed and mounted in weak corrosive sublimate solution a prominence was noticed on the ventral side of the mouth suggestive of the asymmetrical boring tooth of the larvae of the ascarids of fish (Stewart, 1914). Whether this was one of the subventral lips or a separate structure it is not possible to say. No such prominence is visible in specimens mounted in Canada balsam.

(6) Larva from the liver of the rat five days after the first infection and two days after the last. (Mounted in Canada balsam.) (Pl. I, figs. 6–7, 9–12.)

Total length 0·375 mm., maximum breadth (opposite to the end of the oesophagus) 0·021 mm., head to nerve ring 0·044 mm., length of the oesophagus 0·085 mm., length of oesophagus/total length 1/4·4.

The head bears three well marked lips, the oesophagus is sinuous before the nerve ring and is surrounded behind the nerve ring by a ring of cells which probably represent the collar nerve cells of free living nematodes. The ventral gland is not visible in this specimen. The cap of cells which overlies its anterior end can, however, be seen clearly in a mutilated specimen where the oesophageal region has been torn away from the remainder of the body. The oesophagus has retracted towards the head and left this group of cells clearly exposed.

The gland is also clearly distinguishable in sections (Pl. I, figs. 6–7, 9–12). It consists of a large elongated cell lying immediately dorsal to the ventral line. At its anterior end it is in very close contact with the ventral line if not in direct protoplasmic continuity with it. (Pl. I, fig. 9.) It possesses one large nucleus only and the protoplasm is eosinophilic. The anterior pole of the cell fits into a cap of cells of the ventral line. This gland is homologous with the ventral gland of *Ascaris capsularia* R. and the anterior cap of cells with the duct. (Stewart, 1906; Baylis, 1916.)

(7) Larva from the lung of the rat ten days after the first infection and six days after the last. (Pl. I, fig. 5.)

Total length 0·661 mm., maximum breadth 0·029 mm., head to nerve ring 0·051, length of the oesophagus 0·13 mm., length of oesophagus/total length 1/5·08.

The three lips are distinct but low, the nerve ring is difficult of recognition, the ventral gland is very distinct, the intestine is open throughout and the anus is patent. A group of cells in the ventral line at the commencement of the posterior third of the body probably represents the rudiment of the female gonads.

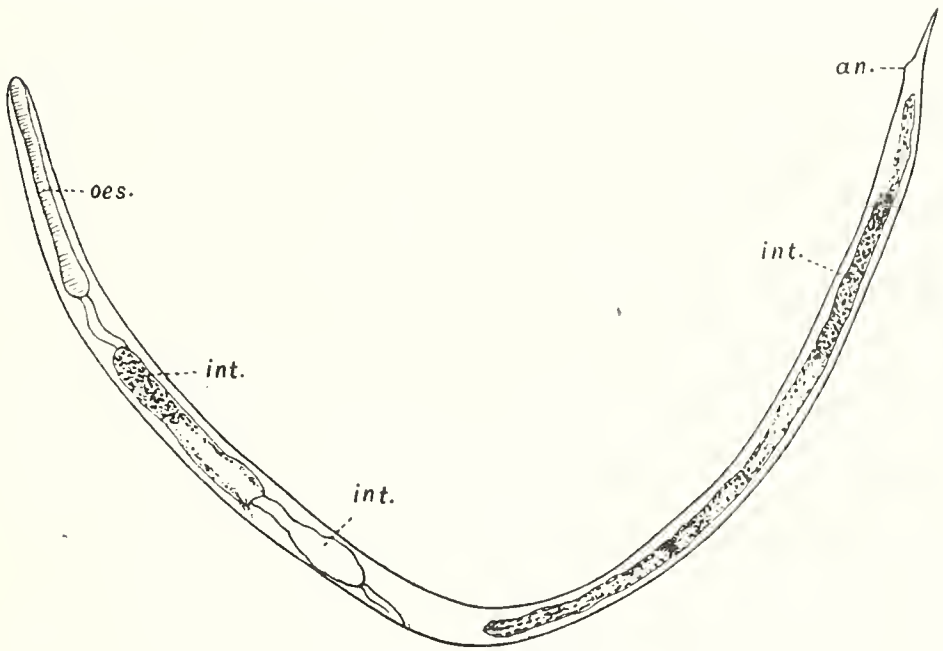

Fig. 6. Larva from the stomach of rat *F*. × 136.

(8) Larvae from the alimentary canal of the rat twelve days after the first infection and nine days after the last.

(*a*) From the stomach (Text-fig. 6). (Mounted in Canada balsam.)

Total length 1·3 mm., maximum breadth 0·037 mm., length of oesophagus 0·2 mm., length of oesophagus/total length 1/6·5.

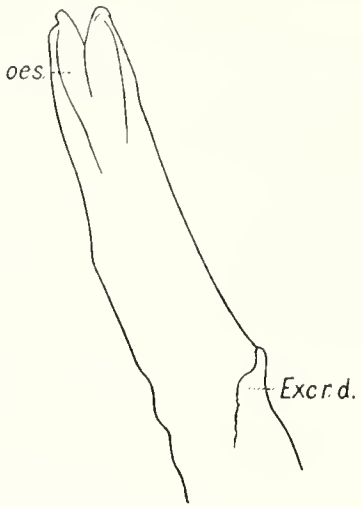

Fig. 7. Head of larva from the rectum of rat *F.* ×525.

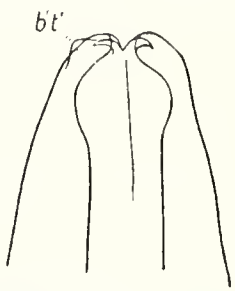

Fig. 8. Head of larva from the lung of rat *C.* ×680.

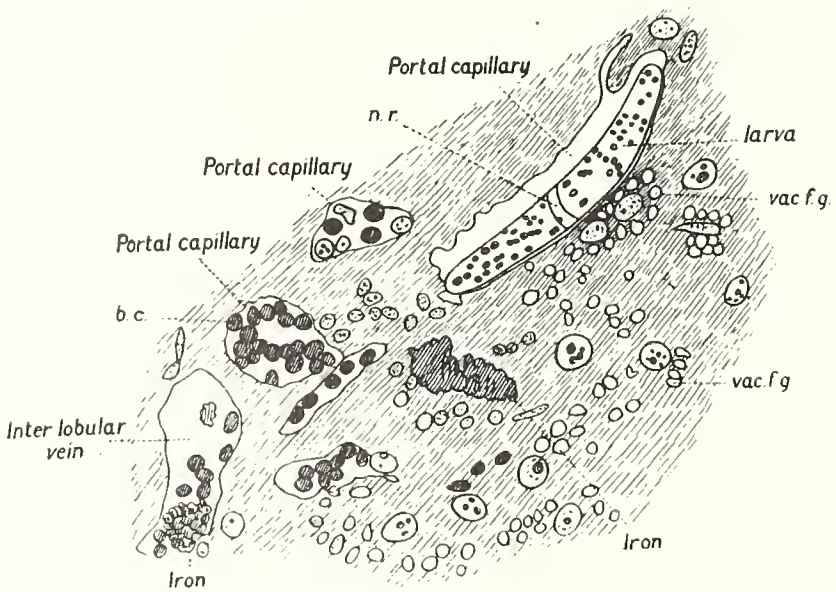

Fig. 9. Section through the liver of mouse *A.* ×510.

The specimen was dead and somewhat degenerated. No lips are visible. The oesophagus resembles that of *Ascaris lumbricoides* larva. The intestine is loaded with black pigment granules.

Text-fig. 6 should be compared with Text-fig. 5. It will be seen that there is a marked general resemblance. The reduction in breadth in the specimen from the stomach is probably due to shrinkage after death and before fixation and to passage through alcohols.

(*b*) From the rectum (Text-fig. 7). This specimen was also dead and was partially digested. In size and general appearance it strongly resembles the preceding. The head bears three well developed lips, one dorsal, two subventral. The duct of the ventral gland is very clearly visible containing a deposit of haematoxylin.

There can be little doubt that this is an *Ascaris* larva.

Summary of Results.

When eggs of *Ascaris lumbricoides* Lin. or *A. suilla* Duj. containing mature embryos gain entrance to the alimentary canal of the sewer rat, *Mus decumanus*, or the mouse, *M. musculus*, they hatch. It is not possible to say at present in what part of the alimentary canal hatching takes place. (The number of animals suitable for experiment at the disposal of the writer is unfortunately so small that he is unable to devote any of them to the working out of the details of the process.) A certain proportion of the larvae thus liberated escape in the faeces where under suitable circumstances they can live for at least three days. It is, however, probable they ultimately succumb and that this is not a true road of development.

The majority of the larvae gain entrance into the body of the host. The exact point of entrance and the time after hatching at which entrance takes place have not been determined. Some animals show signs of illness on the second day after infection. The time elapsing between infection and the entrance of the larvae into the body is therefore probably not more than two days.

Larvae are found in the lungs and liver of the host not later than four days after infection and possibly as early as two days. Sections of the tissues show that they are situated in the air vesicles of the lung and in the blood capillaries of the liver close to the interlobular branches of the portal vein.

Larvae are not found in the liver after the fifth day from infection. They are found in the bronchi about the seventh day and in the trachea on the eighth day.

No larvae are found in any portion of the lung on the ninth day

after infection. Dead larvae have been found in the stomach and rectum on the ninth day after the last infection.

The route by which the larvae reach these sites is of course not definitely proved but from anatomical considerations it is hardly possible that it can be other than one of the two following (the diameter of the larva is three times that of a red blood corpuscle of the mouse. The larva could therefore not pass through the lumen of an ordinary capillary vessel):

(1) Boring through the wall of the stomach or intestine the larva enters a mesenteric venule and is carried to the liver. It is here arrested at the entrance to the hepatic capillary plexus and it is for this reason that so many larvae are found in the capillaries close to the interlobular veins. The liver undergoes extreme and acute fatty degeneration so that the larvae are able to penetrate along the capillaries between the degenerated columns of liver cells to the hepatic venules. Thence they pass in the hepatic vein to the heart and by the pulmonary artery to the lung. They are of course at once arrested by the pulmonary capillary field. Embolism of the smaller branches of the pulmonary artery takes place with haemorrhage around these arterioles. The larvae readily work their way along with the effused blood into the air vesicles and thence into the bronchi and trachea.

(2) The larva after hatching in the stomach or duodenum travels up the bile duct and reaches the bile capillaries of the interlobular zone. It here bores its way through the degenerated liver tissues and reaching a hepatic venule continues its course as in the first case.

During the residence in the body of the rat or mouse the larvae grow from a length of 0·22 mm. to 1·4 mm. The proportion length of oesophagus/total length diminishes from 1/2·5 to 1/6·1. The ventral line which is the greatest of the longitudinal lines in the embryo is reduced to the same dimensions as the dorsal and lateral lines. The ventral gland (the rudiment of the excretory system) is developed from a cell of the ventral line, enlarges very greatly, acquires the massive nucleus characteristic of *Ascaris* larvae (Stewart, 1906; Baylis, 1916) and finally develops its duct from the cells of the ventral line. The intestine, anal canal and anus become pervious. The rudiment of the female gonads appears.

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DESCRIPTION OF PLATE I.

Fig. 1. Embryo of *Ascaris suilla* Duj. expressed from an egg 40 days old. Stained with haematoxylin, mounted in Canada balsam. ×1020.

Fig. 2. Larva of *Ascaris lumbricoides* Lin. from liver of mouse *A*. Stained with haematoxylin, mounted in Canada balsam. ×787.

Fig. 3. Larva of *Ascaris lumbricoides* Lin. from lung of rat *G*. Stained with haematoxylin, mounted in Canada balsam. ×787.

Fig. 4. Section of the lung of rat *C*, showing a larva in an air vesicle. ×510.

Fig. 5. Larva of *Ascaris suilla* Duj. from the lung of rat *D*. Stained with haematoxylin, mounted in Canada balsam. ×510.

Fig. 6. Sagittal section through the region of the ventral gland in a larva of *Ascaris lumbricoides* Lin. in liver of rat *G*. ×787.

Fig. 7. A section 0·006 mm. thick, partly sagittal, partly transverse through the oesophageal region of a larva of *Ascaris lumbricoides* Lin. in the liver of rat *G*. ×1020.

Fig. 8. Section through a bronchus of the rat *D*, showing a larva in the bronchus. ×510.

Fig. 9. Transverse section next to that depicted in Fig. 7 (rat *G*). ×1020.

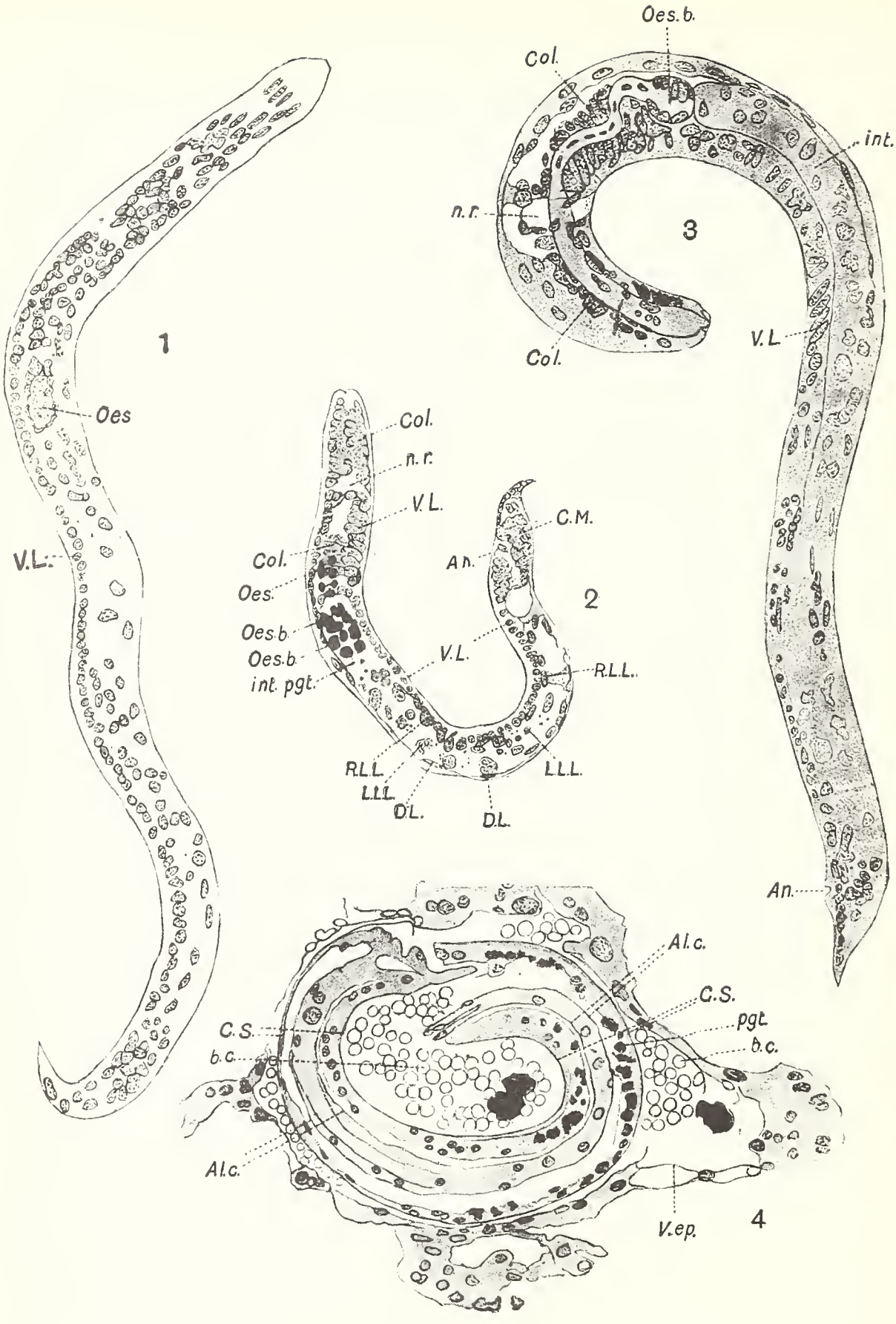
Fig. 10. Transverse section next to the above. ×1020.

Fig. 11. Transverse section next to the above. ×1020.

Fig. 12. Transverse section third in series from the above. ×1020.

REFERENCE LETTERS IN TEXT-FIGURES AND PLATE.

Al.C. alimentary canal. *An.* anus. *An.C.* anal canal. *b.c.* blood corpuscles. *b.t.* boring tooth. *br.ep.* bronchial epithelium. *Col.* circum-oesophageal collar. *C.S.* cuticular surfaces of the worm. *D.Lp.* dorsal lip. *D.L.* dorsal line. *Excr.d.* excretory duct. *go.* gonad. *int.* intestine. *int.gr.* pigment granule of intestine. *L.* larva. *L.L.L.* left lateral line. *L.S.V.L.* left subventral lip. *Mus.* muscle. *n.r.* nerve ring. *n.v.gl.* nucleus ventral gland. *Oes.* oesophagus. *Pgt.* pigment. *R.L.L.* right lateral line. *R.S.V.L.* right subventral line. *S.V.L.* subventral lip. *V.ep.* epithelium of air vesicle. *V.L.* ventral line. *V.gl.* ventral gland. *vac.f.g.* vacuole of fatty degeneration.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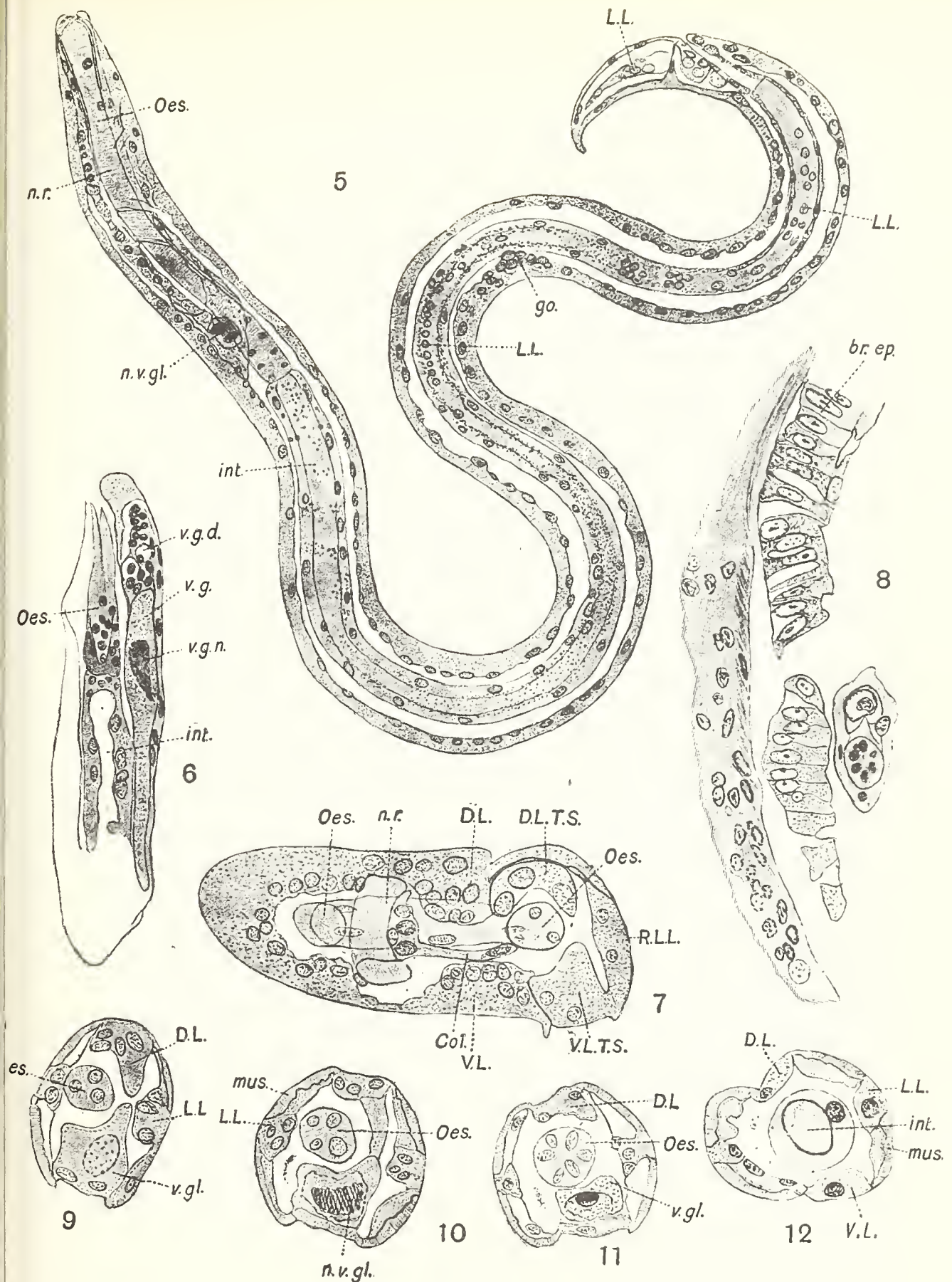
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A CONTRIBUTION TO THE BIONOMICS OF *PEDICULUS HUMANUS* (*VESTIMENTI*) AND *PEDICULUS CAPITIS*.

By A. BACOT,

Entomologist to the Lister Institute of Preventive Medicine.

In its inception this work was planned with a view to supplement our present knowledge of the life history of *Pediculus humanus* (*vestimenti*, which we owe so largely to Warburton[1] (1909). The scheme was to work out in detail certain problems relating to sex and fertility and incidentally to obtain further evidence in support of what is already known concerning the laying and hatching of eggs. The success of an attempt to breed *P. capitis* under conditions that had already proved satisfactory with *P. humanus* suggested that it was worth while enlarging the scope of the scheme in order to include the head louse, as this insect seemed to be amenable to the same conditions of captivity.

These conditions, as nearly natural for *P. humanus* as were consistent with captivity and isolation, are admittedly more artificial when applied to *P. capitis*; it is necessary to keep this fact in mind when comparing the bionomics of the two insects as described in this paper.

The lower egg production of *capitis* is in all probability chiefly due to the smaller egg-containing capacity of its body; for, in spite of the fact that the eggs of the head louse are slightly smaller than those of the species associated with clothing, the body extension of the ♀♀ *P. humanus* still gives them a marked advantage with respect to the number of fully developed eggs that they can carry. The shorter life, and apparently lower vitality of *P. capitis* as compared with *P.*

[1] I was unaware of the publication of Sikora's (1915) excellent paper, which contains much fuller details than any hitherto published account of the biology of *P. humanus* (*vestimenti*), until the experiments detailed in this paper had been mapped out and half completed.

humanus, is probably due, at all events in part, to the method of feeding and other conditions of captivity.

Origin of the strains experimented with.

Lice were obtained from three separate sources; a London Borough Infirmary, one of the London County Council cleansing stations, and a Salvation Army shelter. I take this opportunity of recording my thanks to the Officials of these Institutions who so kindly assisted me by providing material.

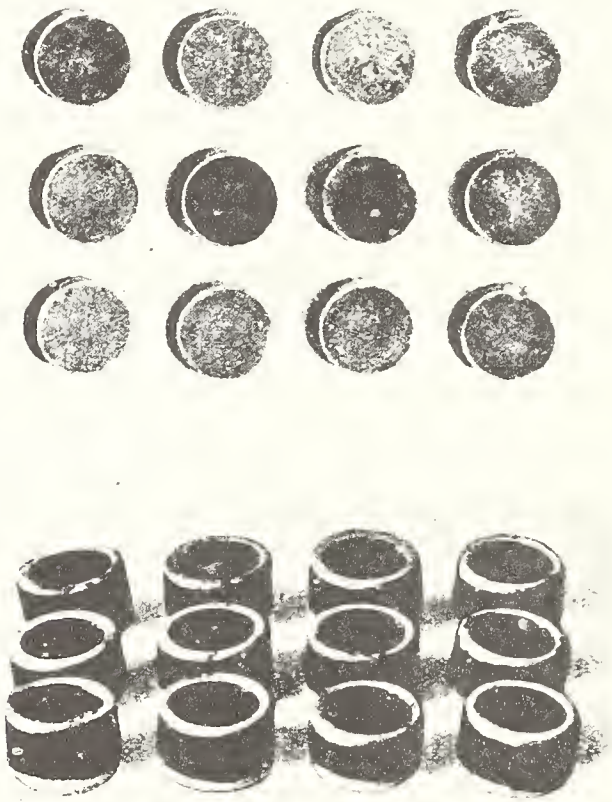
The insects obtained from these sources were placed together and treated as a single stock in the case of *P. humanus*. With *P. capitis*, however, two stocks were kept; one raised from nits on the hair and the other from active insects received with the hair. This course was pursued in order to avoid possible error due to a mixed infection, but as no difference in size, structure, or habits was observed between the two stocks, it was concluded, after several generations had been bred, that only *P. capitis* had been present. Subsequently either stock was drawn upon to supply insects for experiment.

Methods. The method of breeding employed was essentially the same as that adopted by the author when experimenting with fleas (Bacot 1914), in one particular adaptation to the circumstances it closely paralleled a feature of the method mentioned by Sikora (1915), the idea, however, was differently applied.

The insects were kept in glass-bottomed entomological boxes lined with a loose slip of cloth to give foothold. In the case of *P. capitis* a small tuft of hair was also generally added, although it was found not to be essential. The open top of the box was covered with chiffon, the lid being partly closed over it to keep it stretched while it was securely tied in position with fine thread; as an extra precaution against escape the box was nested in one of a larger size. Feeding took place readily through the chiffon when this was placed against a suitable skin surface. As it was necessary during the progress of the research to feed isolated individuals or families in as many as three dozen separate boxes at once, small ones of three quarters of an inch diameter were used. Holes punched in pieces of card into which the gauze covered boxes were inserted, enabled them to be kept in position against the body during sleep, with the aid of a flannel belt. The insects had the option of feeding at any time covering a period of six or seven hours during which the chiffon covered openings of the boxes were against the skin.

During the day the boxes were carried in the pockets of a waistcoat, the eggs laid being hatched in the same or similarly fitted boxes carried beneath the clothing.

The only section of the experiments in which this procedure was modified was in that dealing with the eggs of ♀♀ submitted to differential



Figs. 1 and 2.

Breeding method when a large number of segregated individuals or broods have to be fed at the same time.

feeding (Table VIII) and the trials conducted to test variability in hatching (Table IX), in which it was thought desirable to keep the eggs at a constant temperature. In both these instances the eggs were laid on cloth and placed in glass tubes plugged with cotton-wool, the tubes being kept in a humid incubator having a constant temperature of 31° C.

Habits of the insects noted when reared under the conditions mentioned above.

P. capitis is much the more active insect of the two. In egg laying either species can adapt itself to cloth or hair. *P. capitis* will sometimes lay eggs on cloth, although there are hairs in the box. *P. humanus,* on the other hand, seldom if ever lays on hair while there is cloth in the box and when compelled to do so the females of this species appear to

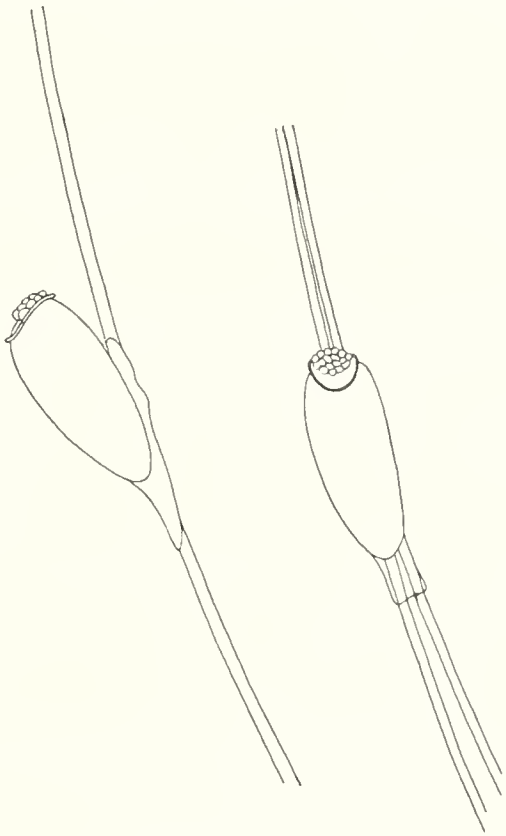


Fig. 3. *P. capitis* × 25.

be less skilful in cementing, or careful in getting the long axis of the egg into alignment with the hair. When forced to lay on hair they not infrequently attach the egg at an angle to the axis of the hair.

Two or three ♀♀ and ♂♂ of *P. humanus* were placed in a small box with human hairs of two or three centimetres long from the forearm. The insects were liberally fed for three days, during this period they laid 35 eggs. Of these one was attached to the side of the box and was also cemented to the hairs which clung together owing to eggs having

been fastened to two or more hairs at the angles of crossing. Nine eggs had been laid on the gauze cover of the box; only four eggs had been attached to the single hairs, while 21 (60 %) of the eggs were cemented to two or more of the hairs, the ♀♀ having apparently searched for positions where the hairs crossed or ran parallel to each other so as to avoid attachment to single hairs.

Several ♀♀ and ♂♂ of *P. capitis* were treated similarly, in this box—36 eggs were laid, all of them on hairs; 28 were attached to a single hair only; six had attachment to two and two to three hairs. A marked

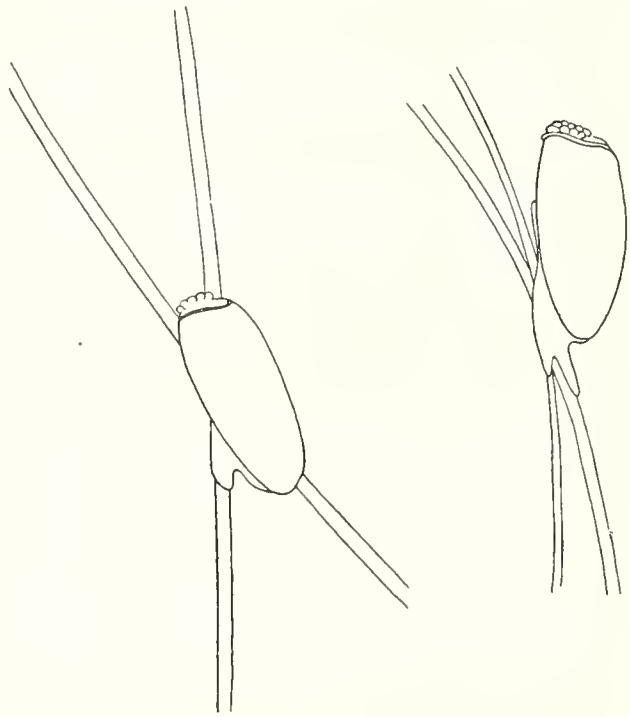


Fig. 4. *P. humanus* × 25.

difference was observable, also, in the selection of position for attachment in the case of the eggs having attachment to more than one hair. In every case the hairs chosen not only ran parallel for more than the length of the egg, but in most cases they were side by side. The parallel hairs affected by *P. humanus* were separated from each other by a well marked space, while in most cases hairs crossing each other at a wide angle had been chosen.

P. humanus ♀♀ in most cases exhibited what may be roughly called a homing instinct; that is to say they returned again and again to the same spot to lay their eggs. As this habit made counting difficult,

various methods of shifting the piece of cloth, turning it, so that the exposed surface went against the side of the box, the addition of a second piece of cloth, etc., were tried in an endeavour to get the eggs spread, instead of clustered. As a rule these attempts were unsuccessful, there seemed to be some attraction which led to the deposit of fresh eggs where others were already laid.

P. capitis ♀♀ occasionally exhibited the same tendency, but it was never so marked or "wilful" as with those of *P. humanus*. Another habit that was marked in the body louse and less noticeable but still to be observed with *P. capitis*, was a tendency to gregariousness shown by clustering, especially on the part of the larvae and nymphs when about to moult.

Pairing may be seen at any period of the day or night on the part of both species, the insects remaining together for a considerable time; periods of over an hour were observed, but I failed to ascertain what the limits were.

Both species show a very definite negative heliotropism, moving towards any shadow or dark coloured object in their vicinity. General observation suggested that their actions differed somewhat, however, according to whether they were at the time of exposure to light resting on a dark or a light surface. The following experiment was carried out with *P. humanus*:

Two eight inch squares of paper, one black and the other white, each having a sufficiently roughened surface to afford good foothold, were placed near together about two feet from a window. The lice at each trial being emptied out of a tube on to the centre of the paper.

Two batches of lice were used, each comprised five females and four males; eight trials were made on each paper—the batches being transposed from black to white and vice versa after each trial.

While these experiments strengthen the idea suggested by my general observations, viz. that there is a difference between the behaviour of the insects dependent upon their being on a light or dark surface when exposed to the light, their movements being less assured in the former case, the result is not so striking as I was led to expect. Had the papers been broader all the active insects would have reached the end farthest from the source of light, as all their tracks towards the sides were diagonals in this direction.

The sluggishness recorded is, with one exception, due to the inactivity of three ♂♂; of the 15 records 14 were due to the inaction of these three individuals.

No. of observation	Paper (black or white)	Distribution of lice on the paper	No. of observation	Paper (black or white)	Distribution of lice on the paper
1	Black	3 *a* 0 *b* 6 *c*	9	Black	1 *a* 1 *b* 7 *c*
2	White	0 *a* 3 *b* 6 *c*	10	White	0 *a* 3 *b* 6 *c*
3	Black	0 *a* 1 *b* 8 *c*	11	Black	0 *a* 0 *b* 9 *c*
4	White	0 *a* 3 *b* 6 *c*	12	White	2 *a* 0 *b* 7 *c*
5	Black	2 *a* 2 *b* 5 *c*	13	Black	3 *a* 0 *b* 6 *c*
6	White	1 *a* 3 *b* 5 *c*	14	White	0 *a* 3 *b* 6 *c*
7	Black	0 *a* 1 *b* 8 *c*	15	Black	0 *a* 1 *b* 8 *c*
8	White	1 *a* 2 *b* 6 *c*	16	White	2 *a* 2 *b* 5 *c*

Totals

Black	9 = 13 %	*a*
	6 = 8 %	*b*
	57 = 79 %	*c*
White	6 = 9 %	*a*
	19 = 26 %	*b*
	47 = 65 %	*c*

NOTE. *a* = lice sluggish and failed to crawl.
b = lice crawled to side of the paper.
c = lice crawled to edge of paper farthest from the source of light.

Length of life of unfed lice under different conditions of temperature.

Lice at all stages of growth were taken from a stock box carried in a vest pocket and submitted to the following conditions: in a room, the air of which is very dry owing to central heating, temperature 16°–18° C.; in a humid incubator at 24·5° C. constant; in a dry air incubator at 37° C. constant.

At 16°–18° C. most of the insects died within four days; two lived five days; one adult was still living on the seventh day.

At 24·5° C. all died within five days.

At 36·1° C. all died within three days.

Newly hatched lice that had not been fed lived less than 24 hours at 36·1° C. When kept unfed in a box in the vest pocket newly hatched lice lived but little more than a day; none survived a second day.

Adults kept in a box unfed in the side pocket of a coat lived five days.

Cold. Active lice in all stages were placed in a cloth-lined box and kept in a cold room at $-2{\cdot}3°$ to $-1{\cdot}1°$ C. for 48 hours; all were stiff and motionless when taken out, but after 24 hours in a vest pocket all revived; they were afforded ample opportunity of feeding, after which they were again kept in the cold room for seven days; all were dead on removal.

Feeding. Hungry lice do not as a rule wander, but settle down to feed at once if placed on a suitable skin area. When a number are placed on a small area of not more than a square inch, there is a considerable difference in the time taken to draw blood; many obtain it within half a minute to a minute, others within two or three minutes, while a few may be five minutes or over. Such delay seldom if ever occurs with bugs (*Cimex lectularius*) presumably owing to their more powerful pumping apparatus, or possibly the greater depth of the wound. Fleas not infrequently fail at the first attempt, in which case they usually shift and try again at another spot. Lice, however, rarely if ever shift, but wait patiently until, presumably, the irritation caused by the injection of saliva dilates the capillaries and brings the flow of blood to the wound. This reliance upon the salivary fluid fits in well with the fact of the more intense and lasting effect of the bite, as compared with that of either fleas or bugs (on the author). With insects having such a generally restricted range of operations there is a very fair chance of their feeding a second time on the same area and benefiting by the inflammation resulting from their previous attacks.

Experiment shows that there is much the same variation in the time required to obtain blood on the part of newly hatched larvae as with larvae in their second skin, nymphs and adults; and the speed with which they fill their crops also varies from about two to fifteen minutes.

Growth and moulting: Pediculus humanus (vestimenti).

Forty newly hatched lice were kept in a box in a vest pocket and afforded opportunity for feeding during six or seven hours each night; cast skins were found as under:

	Number of days after hatching													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Number of cast skins			1	17	22		6	29	5	2	1	22	13	2
			1st moult				2nd moult			3rd moult				
			3 %	42 %	55 %		15 %	72 %	13 %	5 %	3 %	55 %	32 %	5 %

TABLE I. *Fertility of* Pediculus humanus (vestimenti).

Insects in the larval stage were taken from a stock box and reared until maturity in separate boxes. The ♀♀ were kept (approximately 7 hours). The ♂ used for the first pairings matured on 23 Dec. 1915 and died on 24 January 1916 (life kept in separate boxes. None of them hatched.

The boxes containing the ♀♀ and eggs were carried in a waistcoat pocket during the day; at night they were in still close

Reference No. of ♀	Date when ♀ matured	Date when the ♂ was added	Copulation observed	Date of removal of ♂	Days counting from maturity of the ♀ to that on which egg														
					1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16t
1	23. XII. 15	23. XII. 15	—	24. XII. 15	—	—	—	—	8	10	4	6	5	6	7	7	4	10	4	10
2	23. XII. 15	24. XII. 15	—	25. XII. 15	—	—	—	—	5	7	4	7	3	7	8	8	3	7	7	6
3	23. XII. 15	25. XII. 15	26. XII. 15	26. XII. 15	—	—	*2*	—	6	4	5	5	7	4	10	6	7	3	7	6
4	23. XII. 15	26. XII. 15	—	27. XII. 15	—	—	*1*	—	—	11	5	7	6	3	11	8	5	7	3	8
5	23. XII. 15	27. XII. 15	—	28. XII. 15	—	—	*2*	—	*4*	1	—	—	—	—	—	—	—	—	—	—
6	24. XII. 15	28. XII. 15	—	29. XII. 15	—	—	—	*2*	*3*	5	6	5	6	7	10	3	6	7	10	4
7	24. XII. 15	29. XII. 15	29. XII. 15	30. XII. 15	—	—	—	*3*	*3*	*4*	4	5	6	6	5	8	5	6	7	4
8	25. XII. 15	30. XII. 15	—	31. XII. 15	—	—	—	*2*	*3*	*2*	3	4	3	6	2	6	3	2	3	3
9	26. XII. 15	31. XII. 15	1. I. 16	1. I. 16	—	—	—	—	*2*	*3*	3	6	4	5	7	8	6	**2**	5	
10	26. XII. 15	1. I. 16	—	2. I. 16	—	—	*2*	*3*	*5*	*1*	*4*	6	1	5	5	3	6	5	1	
11	28. XII. 15	2. I. 16	—	3. I. 16	—	—	*1*	*3*	*4*	*6*	5	6	6	5	9	9	2	6	5	
12	28. XII. 15	3. I. 16	—	4. I. 16	—	—	—	*1*	*2*	*4*	*4*	3	4	3	7	4	5	3	2	
13	30. XII. 15	4. I. 16	—	5. I. 16	—	—	*1*	*4*	*5*	*3*	7	8	5	8	4	6	10	7	3	
14	1. I. 16	5. I. 16	5. I. 16	6. I. 16	—	*1*	*3*	*4*	*4*	5	6	8	5	6	8	7	6	7	10	
15	6. I. 16	6. I. 16	6. I. 16	10. I. 16	—	—	—	5	3	4	7	2	10	3	8	4	7	8	5	1
16	10. I. 16	10. I. 16	10. I. 16	13. I. 16	—	—	—	3	3	7	8	5	6	6	10	4	6	9	4	
17	13. I. 16	13. I. 16	—	15. I. 16	—	—	3	5	4	5	7	7	4	10	4	7	8	8	2	
18	15. I. 16	15. I. 16	—	19. I. 16	—	—	—	5	7	6	7	8	4	8	6	10	8	6	8	
19	19. I. 16	19. I. 16	19. I. 16	20. I. 16	—	—	—	3	5	5	7	7	4	8	7	6	10	2	10	
20	20. I. 16	20. I. 16	—	22. I. 16	—	—	3	5	5	7	6	8	5	3	8	3	9	8	**6**	
21	22. I. 16	22. I. 16	—	23. I. 16	—	—	1	4	5	6	7	6	7	7	6	10	—	9	6	

NOTE. The figures in heavy type indicate that these eggs were kept in separate boxes from those laid earlie in of laying before this test was made was progressively shortened in order that the opportu ty

Particulars of pairing and egg laying.

nd fed in these separate boxes, the ♂ being placed with each ♀ in rotation. Opportunity for feeding was given each night 2 days). Eggs laid before the introduction of the ♂ are italicised and were, in the case of ♀♀ Nos. 6, 7, 10, 12, 13 and 14,

elation to the human body.

ere laid. The number of eggs is indicated by the figure below																		Total eggs laid per ♀	Remarks
17th	18th	19th	20th	21st	22nd	23rd	24th	25th	26th	27th	28th	29th	30th	31st	32nd	33rd	34th	35th	
8	2	9	2	7	4	5	—	—	—	—	—	—	—	—	—	—	—	—	118	Died on the 23rd day.
5	4	9	5	8	5	6	7	2	5	4	3	5	1	—	1	4	4	—	150	Died on the 34th day.
5	8	7	5	5	4	5	5	7	3	6	4	4	5	3	6	4	6	2	166	See further entries after second pairing, Table II.
10	3	6	6	8	7	5	7	9	5	9	7	5	4	3	3	—	—	—	172	Died on the 32nd day.
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7	Died on the 11th day. Eggs infertile.
5	10	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	95	Lost on the 19th day.
4	6	6	3	4	5	2	9	—	4	4	3	6	—	1	—	—	—	—	123	Died on the 32nd day.
7	3	2	6	2	5	3	1	—	—	—	—	—	—	—	—	—	—	—	70	Accidentally killed on the 23rd day. Eggs infertile.
9	7	5	12	3	2	7	6	7	6	6	5	5	5	4	7	3	—	—	159	See further entries after second pairing, Table II.
4	6	6	5	3	8	5	2	1	6	4	1	—	—	—	—	—	—	—	102	Died on the 29th day.
4	9	7	11	14	7	9	8	6	5	5	7	3	10	—	—	—	—	—	180	Died on the 30th day.
4	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	55	Died on the 19th day.
6	7	8	3	4	4	5	6	4	5	4	6	5	5	4	—	—	—	—	154	See further entries after second pairing, Table II.
7	7	6	6	6	2	3	10	9	6	6	10	6	4	3	7	—	—	—	181	,, ,, ,,
5	4	9	3	7	5	7	8	5	5	4	10	—	—	—	—	—	—	—	148	,, ,, ,,
6	9	8	5	5	6	7	6	7	—	—	—	—	—	—	—	—	—	—	137	,, ,, ,,
9	7	3	6	7	8	6	7	7	—	—	—	—	—	—	—	—	—	—	141	,, ,, ,,
8	5	9	8	8	9	9	7	—	—	—	—	—	—	—	—	—	—	—	152	,, ,, ,,
8	5	8	5	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	115	,, ,, ,,
8	7	6	8	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	117	,, ,, ,,
13	1	10	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	110	None of these eggs hatched, presumably this ♀ was not fertilized as she laid fertile eggs after pairing with another ♂, see entries on Table II.

rder to test if the ♀♀ still retained the power of fertilizing them. It will be noted that with experience the period f a second pairing might take place before the ♀ was too old and feeble to profit by it.

Table I gives the record of 21 ♀♀ of *P. humanus* which were segregated in the larval stage and on reaching maturity were placed in rotation with a single ♂. Of these 21 ♀♀ 18 were more or less effectively fertilized by the one ♂. It will be noted that the ♀♀ invariably commenced oviposition irrespective of their having paired; probably egg development is entirely a question of nutrition and laying is completely automatic while feeding continues at a favourable temperature. In no case did eggs laid by virgin ♀♀ hatch.

As regards the three ♀♀ which laid only infertile eggs—it is probable that the death of No. 5 was connected with pairing, as she laid but one egg after the introduction of the ♂, and she died with the whole body and limbs as far as claws tinged with red. I suspect that death resulted from rupture of the alimentary canal due to violence during the sexual act. Deaths showing a similar post-mortem appearance are by no means uncommon. In the course of the experiments it was remarked that death frequently followed, if it did not actually occur during, the act of pairing if the ♀♀ were nearing their age limit.

In the case of No. 8 it is doubtful if pairing ever took place; this ♀ proved a poor egg layer, but the evidence available from the other ♀♀ which laid prior to pairing precludes any suggestion that low egg production resulted from infertility; it is possible, however, that both the failure to pair and small egg laying capacity were the outcome of a low vitality.

Probably the ♂ was too feeble to pair with No. 21, as he died the following day. Table V shows that there was no question in regard to the vitality of this ♀ as she laid fertile eggs after she had been placed with another ♂.

It was obvious early in the course of the experiment that ability of the ♀♀ to lay fertilized eggs after the removal of the ♂ did not continue for life (see Table IV). In order to test the period, the ♀♀ were removed to fresh boxes at a progressively shortened interval of time. The eggs laid in the second box are indicated by printing the figures in heavy type. By this means a general indication of the duration of fertility was attained; in the cases where eggs hatched which had been laid in the second box, showing definitely that fertility was still retained, the term "at least" indicates that the number of days might have been more. In several cases the number of days has been calculated by counting the number of eggs laid in sequence up to the number which hatched and reckoning the number of days from the removal of the ♂ up to the date of the last laid egg included in the total. In both cases it is probable that

TABLE II.

Pediculus humanus (vestimenti). *Particulars of egg laying after a second ♂ had been placed with the ♀♀.*

A ♂ that matured on the 1st January, 1916 was placed with ♀ No. 3 on the 26th January, he was afterwards placed with ♀♀ Nos. 9. 13, 14, 15 and 16, he was lost on the 5th February 1916. A ♂ taken from the stock box, of uncertain age, was placed with the later ♀♀ Nos. 17—21.

Reference number of ♀	Day, counting from maturity of ♀, when the second ♂ was introduced	Date on which the second ♂ was introduced	Day of copulation when observed	Day when ♂ was removed	Day, counting from maturity of ♀, on which eggs were laid after the introduction of the second ♂																									Total eggs laid per ♀	Remarks
					20th	21st	22nd	23rd	24th	25th	26th	27th	28th	29th	30th	31st	32nd	33rd	34th	35th	36th	37th	38th	39th	40th	41st	42nd	43rd	44th		
3	35th	26. I. 16	35th	36th	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6	4	3	—	—	—	—	—	—	13	Died 38th day.
9	33rd	27. I. 16	35th	35th	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	4	,, 35th ,, whilst paired
13	31st	29. I. 16	—	34th	—	—	—	—	—	—	—	—	—	—	—	—	3	2	—	—	—	—	—	—	—	—	—	—	—	5	,, 33rd ,, eggs infertile.
14	32nd	1. II. 16	—	33rd	—	—	—	—	—	—	—	—	—	—	—	—	—	5	7	6	7	5	7	5	4	10	—	—	—	56	,, 41st ,, ,, ,,
15	28th	2. II. 16	—	29th	—	—	—	—	—	—	—	—	—	6	6	5	4	3	5	4	4	1	—	—	—	—	—	—	—	38	,, 37th ,, ,, ,,
16	25th	3. II. 16	—	27th	—	—	—	—	—	—	6	10	6	4	9	6	6	4	10	7	8	4	—	—	—	—	—	—	—	80	Placed with a third ♂ on 37th day and fertile eggs laid see Table III.
17	25th	6. II. 16	—	26th	—	—	—	—	—	—	7	9	3	10	8	7	4	8	4	7	6	2	7	3	—	10	3	5	5	108	Died 44th day.
18	24th	7. II. 16	—	25th	—	—	—	—	—	8	9	8	9	6	8	8	5	7	8	9	7	4	8	—	8	11	7	—	—	130	Placed with a third ♂ on 42nd day, see Table III.
19	21st	8. II. 16	21st	22nd	—	—	7	7	8	6	6	7	5	7	3	2	—	—	—	—	—	—	—	—	—	—	—	—	—	58	Died 31st day.
20	21st	9. II. 16	—	22nd	—	—	7	6	8	5	6	4	10	7	8	7	9	7	6	3	3	6	4	2	—	—	—	—	—	108	,, 39th ,,
21	19th	10. II. 16	20th 23rd	24th	—	7	7	3	11	4	6	11	5	5	7	10	10	8	—	—	—	—	—	—	—	—	—	—	—	94	,, 33rd ,,

TABLE III.

Pediculus humanus (vestimenti). *Particulars of egg laying after a third ♂ had been placed with the ♀♀.*

♂♂ taken from stock were used, age uncertain.

Reference number of ♀	Day, counting from maturity of ♀, when the third ♂ was introduced	Date on which the third ♂ was introduced	Day of copulation when observed	Day when ♂ was removed	Day, counting from maturity of the ♀, on which eggs were laid after the introduction of the third ♂								Total eggs laid per ♀	Remarks
					38th	39th	40th	41st	42nd	43rd	44th	45th		
16	37th	15. II. 16	—	not removed up to time of females death	3	1	—	—	—	—	—	—	4	Died 39th day.
18	42nd	25. II. 16	—		—	—	—	—	—	2	8	3	13	Died 46th day, eggs infertile.

TABLE IV. *Fertility of* Pediculus humanus (vestimenti).

NOTE. There is no necessary relation between the lice emerging on any particular day and the eggs laid on other; they were kept in the box with the laying ♀ until she was transferred to a new box. The zero

Reference number of ♀	Date when ♀ matured	Date when the ♂ was added	Days counting from date on which the ♂ was																	
			7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th	21st	22nd	23rd	24th	25th
1	23. XII. 15	23. XII. 15	—	—	—	—	1	1	3	3	3	7	4	5	6	9	8	6	4	—	—
2	23. XII. 15	24. XII. 15	—	—	—	—	—	1	3	4	6	3	7	6	7	5	6	4	5	—	1
3	23. XII. 15	25. XII. 15	—	—	—	—	—	3	2	7	5	6	6	7	5	7	5	5	4	10	7
4	23. XII. 15	26. XII. 15	—	—	—	—	—	3	8	5	6	2	6	6	11	5	3	1	—	—	—
5	23. XII. 15	27. XII. 15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6	24. XII. 15	28. XII. 15	—	—	—	—	—	2	4	6	4	8	12	7	8	3	2	—	1	—	—
7	24. XII. 15	29. XII. 15	—	—	—	—	1	3	3	5	4	6	5	8	2	4	9	6	8	—	1
8	25. XII. 15	30. XII. 15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9	26. XII. 15	31. XII. 15	—	—	—	—	—	—	2	8	8	7	2	9	2	8	11	5	4	7	9
10	26. XII. 15	1. I. 16	—	—	—	—	2	3	1	—	3	2	6	3	3	1	—	—	—	—	—
11	28. XII. 15	2. I. 16	—	—	—	—	4	11	4	4	7	7	6	8	5	7	7	12	7	7	2
12	28. XII. 15	3. I. 16	—	—	—	—	—	1	5	4	3	9	3	1	1	1	—	1	—	—	—
13	30. XII. 15	4. I. 16	—	—	—	—	1	10	—	7	9	5	8	2	4	7	6	2	2	—	—
14	1. I. 16	5. I. 16	—	—	—	—	—	5	5	5	8	9	4	4	4	7	8	4	4	3	—
15	6. I. 16	6. I. 16	—	—	—	—	—	1	1	3	5	2	6	5	7	5	7	9	6	4	6
16	10. I. 16	10. I. 16	—	—	—	—	—	—	1	2	7	4	7	8	8	6	5	9	9	9	4
17	13. I. 16	13. I. 16	—	—	—	—	—	1	3	7	5	5	7	7	5	6	7	10	4	8	2
18	15. I. 16	15. I. 16	—	—	—	—	—	—	2	5	7	6	6	7	13	7	10	8	1	1	—
19	19. I. 16	19. I. 16	—	—	—	—	—	—	—	5	3	8	8	12	1	9	12	6	7	**3**	**1**
20	20. I. 16	20. I. 16	—	—	—	—	1	1	6	3	7	8	7	5	7	9	4	—	—	—	—
21	22. I. 16	22. I. 16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Figures in heavy type refer to cases in

Particulars of the hatching of the eggs.

any particular date. It was found impracticable to keep the eggs laid on different days separate from each date from which the number of days has been reckoned is the date on which the ♂ was placed with the ♀.

placed with the ♀										Total of eggs laid per ♀	Total of eggs hatched	Percentage of possible fertile eggs	Remarks
26th	27th	28th	29th	30th	31st	32nd	33rd	34th	35th				
—	—	—	—	—	—	—	—	—	—	118	60	51 %	Ability to fertilize eggs retained for *less* than 20 days.
1	—	—	—	—	—	—	—	—	—	150	59	39 %	,, ,, ,, ,, 24 ,,
4	7	5	1	—	1	—	—	—	—	166	97	59 %	,, ,, ,, *more* ,, 19 ,,
—	—	—	—	—	—	—	—	—	—	172	56	33 %	,, ,, ,, *less* ,, 19 ,,
—	—	—	—	—	—	—	—	—	—	7	0	nil	It is doubtful if any pairing took place.
—	—	—	—	—	—	—	—	—	—	95	57	63 %	Ability to fertilize eggs retained for *at least* 10 ,,
—	—	—	—	—	—	—	—	—	—	123	65	57 %	,, ,, ,, *less* than 21 ,,
—	—	—	—	—	—	—	—	—	—	70	0	nil	It is doubtful if any pairing took place.
9	3	2	4	5	5	4	—	—	1	159	115	75 %	Ability to fertilize eggs retained for *at least* 20 ,,
—	—	—	—	—	—	—	—	—	—	102	24	28 %	,, ,, ,, ,, 6 ,,
—	—	—	1	—	—	—	—	—	—	180	99	60 %	,, ,, ,, ,, 18 ,,
—	—	—	—	—	—	—	—	—	—	55	29	66 %	,, ,, ,, ,, 7 ,,
—	—	—	—	—	—	—	—	—	—	154	63	46 %	,, ,, ,, *not more* than 18 ,,
—	—	—	—	—	—	—	—	—	—	181	70	41 %	,, ,, ,, ,, 20 ,,
9	3	10	1	1	4	1	—	—	—	148	96	65 %	,, ,, ,, *over* 16 ,,
2	5	1	1	—	—	—	—	—	—	137	88	64 %	,, ,, ,, ,, 15 ,,
—	—	—	—	—	—	—	—	—	—	141	77	55 %	,, ,, ,, *not more* than 19 ,,
—	—	—	—	—	—	—	—	—	—	152	73	48 %	,, ,, ,, ,, 17 ,,
1	1	—	—	—	—	—	—	—	—	115	77	67 %	,, ,, ,, *about* 15 ,,
—	—	—	—	—	—	—	—	—	—	117	58	50 %	,, ,, ,, *not more* than 12 ,,
—	—	—	—	—	—	—	—	—	—	110	0	nil	It is doubtful if any pairing took place with this ♂, see entries under Table V.

which eggs laid in the second box hatched.

TABLE V.

Pediculus humanus (vestimenti). *Particulars of hatching of the eggs laid after the second ♂ had been placed with ♀♀.*

Reference number of ♀	Day counting from maturity of the ♀ when the second ♂ was introduced	Days counting from the date on which the second ♂ was placed with the ♀																	Total of eggs laid per ♀	Total of eggs hatched	Percentage fertile	Remarks
		10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th	21st	22nd	23rd	24th	25th	26th	27th				
3	35th	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	13	2	15 %	Died 38th day.
9	33rd	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	3	75 %	,, 35th ,, whilst paired.
13	31st	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	0	0	,, 33rd ,, eggs infertile.
14	32nd	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	56	0	0	,, 41st ,, ,, ,,
15	28th	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	38	0	0	,, 37th ,, ,, ,, .
16	25th	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	80	0	0	Placed with a third ♂ on 37th day and fertile eggs laid subsequently, see Table VI.
17	25th	—	—	8	6	5	5	7	6	6	4	3	1	—	—	—	—	—	—	108	51	47 %	Died 44th day.
18	24th	—	6	10	6	6	8	6	6	7	8	3	8	5	6	4	3	—	—	130	92	71 %	Placed with a third ♂ on 42nd day, see Table VI.
19	21st	—	3	7	6	6	7	2	3	5	1	5	—	—	—	—	—	—	—	58	45	78 %	Died 31st day.
20	21st	—	4	5	9	6	4	7	6	8	4	8	6	4	5	3	5	1	2	108	87	81 %	,, 39th ,,
21	19th	—	1	5	7	7	6	3	5	3	3	7	5	5	4	5	3	4	—	94	73	78 %	,, 33rd ,.

TABLE VI.

Pediculus humanus (vestimenti). *Particulars of hatching of the eggs after the third ♂ had been placed with ♀♀.*

Reference number of ♀	Days counting from maturity of the ♀ when the third ♂ was introduced	Days counting from the date on which the third ♂ was placed with the ♀						Total of eggs laid per ♀	Total of eggs hatched	Percentage fertile	Remarks
		10th	11th	12th	13th	14th	15th				
16	37th	—	—	—	2	—	1	4	3	75 %	Died 39th day.
18	42nd	—	—	—	—	—	—	13	0	0	,, 46th ,, eggs infertile.

TABLE VII.

Pediculus humanus (vestimenti). *Summary of Tables I to VI.*

Reference number of ♀	Length of life in days	Number of days between maturity and pairing	Number of days between maturity and oviposition	Number of eggs laid before the introduction of a ♂	Number of eggs laid, pairing No. 1	Approximate period of fertility in days	Percentage of fertile eggs	Number of eggs laid, pairing No. 2	Approximate period of fertility in days	Percentage of fertile eggs	Number of eggs laid, pairing No. 3	Approximate period of fertility	Percentage of fertile eggs	Total number of eggs laid	Average number laid each day	Total number of eggs hatched	Percentage of *possibly fertile* eggs which hatched
1	23	1	1	3	118	less than 20	51 %	—	—	—	—	—	—	118	5·3	60	51 %
2	34	no choice	4	—	150	,, ,, 24	39 %	—	—	—	—	—	—	150	4·4	59	59 %
3	38	,,	2	2	166	more than 19	59 %	13	—	15 %	—	—	—	179	4·7	99	56 %
4	32	,,	2	1	172	less than 19	33 %	—	—	—	—	—	—	172	5·4	56	33 %
5	11	,,	2	6	7	infertile	nil	—	—	—	—	—	—	7	·6	nil	nil
6	19 (lost)	,,	3	5	95	at least 10	63 %	—	—	—	—	—	—	95	5·0	57	63 %
7	32	,,	3	10	123	less than 21	57 %	—	—	—	—	—	—	123	3·9	65	57 %
8	23 (killed)	,,	3	7	70	infertile	nil	—	—	—	—	—	—	70	3·0	—	nil
9	35	,,	4	5	159	at least 20	75 %	4	—	75 %	—	—	—	163	5·0	118	75 %
10	29	,,	2	15	102	,, ,, 6	28 %	—	—	—	—	—	—	102	3·5	24	28 %
11	30	,,	2	14	180	,, ,, 18	60 %	—	—	—	—	—	—	180	6·0	99	60 %
12	19	,,	3	11	55	,, ,, 7	66 %	—	—	—	—	—	—	55	2·9	29	66 %
13	33	,,	2	13	154	not more than 18	46 %	5	infertile	nil	—	—	—	159	4·8	63	43 %
14	41	,,	1	12	181	,, ,, 20	41 %	56	,,	,,	—	—	—	237	5·8	70	31 %
15	37	less than 1	3	—	148	over 16	65 %	38	,,	,,	—	—	—	186	5·0	96	52 %
16	39	,, ,, 1	3	—	137	,, 15	64 %	80	,,	,,	4	1 day	75 %	221	5·6	91	41 %
17	44	doubtful	2	—	141	not more than 19	55 %	108	at least 8	47 %	—	—	—	249	5·7	128	51 %
18	46	,,	3	—	152	,, ,, 17	48 %	130	,, ,, 13	71 %	13	infertile	nil	295	6·4	165	56 %
19	31	less than 1	3	—	115	about 15	67 %	58	,, ,, 7	78 %	—	—	—	173	5·8	122	71 %
20	39	doubtful	2	—	117	not more than 12	50 %	108	,, ,, 13	81 %	—	—	—	225	5·8	145	64 %
21	33	1	2	—	110	infertile	nil	94	,, ,, 11	78 %	—	—	—	204	6·2	73	36 %

Life of ♂ 32 days. Longest ♀ life 46 days. Average ♀ life 34 days. Number of ♀♀ fertilized by one ♂, 18. Eggs laid by unfertilized ♀♀ did not hatch. The period of fertility lasts about 12 to 21 days. The number of eggs fertilized during the period of fertility probably depends on the rate of egg laying, the highest record was 115 by ♀ No. 9. The egg laying capacity of normal ♀♀ is certainly conditioned by food (see Table VIII) and by temperature as pointed out by Sikora (1915). In the above experiment (omitting ♀ No. 5) the numbers range from 55 to 295 with an average of 177 and a daily average of 5·1.

TABLE VIII.

The influence of food supply upon (a) the number of eggs laid and (b) their fertility.

Method. A number of lice in the larval stage were reared until adult. 5 ♂♂ and 5 ♀♀ were then placed in each of two boxes. Those in box B were fed at night only, period for feeding available about 7 hours. In box A additional feeding time, 2 or 3 hours, was given by day. Boxes kept in a breast pocket by day. (A false start was made owing to a ♀ in one of the boxes escaping during the counting of the eggs; as the escape was not noticed until the 10th day a fresh start was made on the 11th day with four pairs in each box.)

Box A.

	Number of eggs laid each day. Figures at top indicate days in the life of the ♀								Number of eggs hatching, each day's laying treated as a separate batch							
	12	13	14	15	16	17	18	19	12	13	14	15	16	17	18	19
Number of eggs laid	32	25	66		34	35	34	30	30	23	62		32	30	30	26

Total 256 = a daily average of 8 per ♀ — Total 233

Percentage hatching = 91 %.

Box B.

	Number of eggs laid each day. Figures at top indicate days in the life of the ♀								Number of eggs hatching, each day's laying treated as a separate batch							
	12	13	14	15	16	17	18	19	12	13	14	15	16	17	18	19
Number of eggs laid	28	17	49		30	22	28	27	28	17	49		29	21	25	26

Total 201 = a daily average of 6·3 per ♀ — Total 195

Percentage hatching = 97 %.

Reverse order of feeding.

On the 20th and 21st days the insects in both boxes were treated alike as regards feeding (at night only) and no records of egg laying were made. On the 22nd and following days the insects were fed in the reverse order; A one period of about 7 hours at night, B two periods 7 hours at night and 2 or 3 hours during the day. On the 26th day a ♀ in box A died and one was also removed from box B. On the 28th day the insects in box B were not fed during the day and both boxes were removed from pocket during that day.

Box A.

	Number of eggs laid each day. Figures at top indicate days in the life of the ♀								Number of eggs hatching, each day's laying treated as a separate batch							
	23	24	25	26	27	28	29	30	23	24	25	26	27	28	29	30
Number of eggs laid	18	18	15	18	17	28		14	17	17	13	16	14	28		12

Total 128 = a daily average of 4·5 per ♀ — Total 117

Percentage hatching = 91 %.

Box B.

	Number of eggs laid each day. Figures at top indicate days in the life of the ♀								Number of eggs hatching, each day's laying treated as a separate batch							
	23	24	25	26	27	28	29	30	23	24	25	26	27	28	29	30
Number of eggs laid	32	30	28	25	23	28		21	31	30	24	23	20	27		21

Total 187 = a daily average of 6·6 per ♀ — Total 176

Percentage hatching = 94 %.

TABLE IX.

Hatching of eggs of Pediculus humanus *when kept at a constant temperature.*

The eggs laid by 10 pairs of newly-matured lice were taken from the boxes in which they were laid (on cloth) each day and kept as separate batches in a humid atmosphere at 30° C.

Number of days counting from the approximate date on which the insects matured	Total number of eggs laid	Record of hatching: Number of days reckoning from the date of laying												Total number of eggs which hatched	Percentage of eggs which hatched
		1	2	3	4	5	6	7	8	9	10	11	12		
1															
2	29	—	—	—	—	—	—	4	12	5	—	—	—	21	72 %
3	60	—	—	—	—	—	—	3	38	6	—	—	—	47	78 %
4	68	—	—	—	—	—	—	2	48	15	1	—	—	66	94 %
5	71	—	—	—	—	—	—	—	24	18	16	—	—	58	82 %
6	84	—	—	—	—	—	—	—	56	13	9	—	—	78	93 %
7 8	120	—	—	—	—	—	—	4	52	45	13	—	—	114	95 %
9	61	—	—	—	—	—	—	—	26	26	—	—	—	52	85 %
10	71	—	—	—	—	—	—	—	21	40	5	—	—	66	93 %
11	62	—	—	—	—	—	—	—	24	24	12	—	—	60	97 %
12	60	—	—	—	—	—	—	—	34	18	6	—	—	58	97 %
13	42	—	—	—	—	—	—	2	29	8	1	—	—	40	95 %
14 15	115	—	—	—	—	—	—	9	68	29	5	—	—	111	97 %
16	64	—	—	—	—	—	—	—	15	36	10	—	—	61	95 %
17	57	—	—	—	—	—	—	—	14	32	4	1	—	51	89 %
18	62	—	—	—	—	—	—	—	20	32	2	1	—	55	89 %
19	57	—	—	—	—	—	—	—	13	16	22	1	—	52	91 %
20 21 22	No record														
23	50	—	—	—	—	—	—	1	40	7	—	—	—	48	96 %
24	48	—	—	—	—	—	—	—	30	16	1	—	—	47	98 %
25	43	—	—	—	—	—	—	—	24	13	—	—	—	37	86 %
26	43	—	—	—	—	—	—	1	25	13	—	—	—	39	91 %
27	40	—	—	—	—	—	—	—	27	7	—	—	—	34	85 %
28 29	56	—	—	—	—	—	—	6	45	4	—	—	—	55	98 %
30	35	—	—	—	—	—	—	4	29	—	—	—	—	33	94 %
	1398							36	714	423	107	3		1283	92 %

Summary

3 % hatched on the 7th day
56 % " " 8th "
33 % " " 9th "
8 % " " 10th "
0·2 % " " 11th "

TABLE X. *Fertility of* Pediculus capitis.

Insects in the second larval skin were taken from a stock box and reared until maturity in separate boxes. Opportunity for feeding was given each night (approximately 7 hours). Owing to the early death of the ♂♂ indicated by the figures 1, 2, 3, 4 in the first column. ♂ No. 1 matured on the 5th January and died on the on the 25th January and died on the 9th February, life 15 days. The last ♂ No. 4 matured on the 9th February capacity of an unfertilized ♀. Eggs laid before the introduction of a ♂ are italicised. The boxes containing

Reference number of ♂	Reference number of ♀	Date when ♀ matured	Date when the ♂ was added	Copulation observed	Date of removal of the ♂	Days counting from the maturity of the ♀ to that on which the eggs																
						1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	19th	20th
No. 2	1	4. I. 16	17. I. 16	—	—	–	*1*	*2*	*3*	*2*	*5*	*2*	*4*	*2*	*3*	*2*	*2*	*4*	2	4	1	1	–	–
No. 1	2	5. I. 16	6. I. 16	—	9. I. 16	–	–	2	3	3	2	6	3	2	6	4	2	5	5	8	1	3	7	–
No. 1	3	9. I. 16	9. I. 16	— died	12. I. 16	–	2	2	5	6	3	5	5	5	7	4	1	4	3	6	1	4	7	5
No. 2	4	13. I. 16	13. I. 16	—	16. I. 16	–	–	3	2	3	3	4	2	3	4	5	2	3	8	7	4	–	1	6
No. 2	5	16. I. 16	16. I. 16	— died	17. I. 16	–	–	3	1	5	2	5	2	5	2	–	–	–	–	–	–	–	–	–
No. 3	6	27. I. 16	27. I. 16	—	31. I. 16	–	–	2	3	3	5	7	5	4	6	7	4	4	6	3	6	–	1	–
No. 3	7	31. I. 16	31. I. 16	—	5. II. 16	–	3	3	4	4	5	6	3	5	5	8	3	–	2	4	8	8	7	–
No. 3	8	5. II. 16	5. II. 16	—	8. II. 16	–	3	4	5	4	6	4	2	9	4	8	5	4	7	7	8	7	7	–
No. 3	9	8. II. 16	8. II. 16	— died	9. II. 16	–	1	6	4	2	7	2	6	5	4	4	4	5	6	4	3	4	5	1
No. 4	10	8. II. 16	10. II. 16	—	11. II. 16	–	–	3	3	3	4	4	4	4	4	7	2	4	5	4	3	5	3	5
No. 4	11	11. II. 16	11. II. 16	—	12. II. 16	–	4	4	5	5	4	8	7	6	3	6	3	5	5	4	5	7	2	4
No. 4	12	12. II. 16	12. II. 16 15. II. 16	—	13. II. 16 16. II. 16	–	–	1	1	2	3	4	4	2	4	5	3	4	3	–	–	–	–	–
No. 4	13	13. II 16	14. II. 16	—	15. II. 16	–	–	3	3	2	5	4	6	1	3	4	4	5	5	–	4	2	6	3
No. 4	14	16. II. 16	16. II. 16	—	18. II. 16	–	–	3	3	2	5	4	4	5	3	3	6	7	3	4	6	6	2	–
No. 4	15	16. II. 16	18. II. 16	—	19. II. 16	–	*3*	3	7	1	3	6	3	7	3	5	1	7	4	5	3	–	5	3
No. 4	16	19. II. 16	19. II. 16 25. II. 16	20. II. 16	21. II. 16 26. II. 16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
No. 4	17	19. II. 16	21. II. 16	—	23. II. 16	–	–	–	2	3	4	4	4	–	5	3	6	5	3	2	7	1	6	5
No. 4	18	23. II. 16	23. II. 16	—	25. II. 16	–	–	5	1	5	4	4	2	2	2	6	6	5	6	5	–	7	3	2
No. 4	19	26. II. 16	26. II. 16	—	1. III. 16	–	2	4	4	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
No. 4	20	1. III. 16	1. III. 16	—	6. III. 16	–	1	3	3	3	3	2	6	5	2	3	–	–	–	–	–	–	–	–
No. 4	7	—	6. III. 16	7. III. 16	8. III. 16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
No. 4	9	—	8. III. 16	—	9. III. 16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
No. 4	10		9. III. 16	— died	10. III. 16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

NOTE. The figures in heavy type indicate that these eggs were kept in separate

Particulars of pairing and egg laying.

The ♀♀ were kept and fed in the boxes in which they matured, the ♂♂ being placed with them in rotation. The first three attempts were of doubtful value and a fourth was initiated with better success. These ♂♂ are 2th, life 7 days. ♂ No. 2 matured on the 12th January and died on the 24th, life 12 days. ♂ No. 3 matured nd died on the 10th March, life 30 days. ♀ No. 1 was purposely kept unpaired for 12 days to test the egg laying he ♀♀ and eggs were carried in a waisteoat pocket during the day, at night they were against the body.

were laid. The number of eggs laid is indicated by the figure below																	Total eggs laid per ♀	Remarks
21st	22nd	23rd	24th	25th	26th	27th	28th	29th	30th	31st	32nd	33rd	34th	35th	36th	37th		
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	40	Died on the 17th day.
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	62	,, ,, 22nd ,,
5	–	2	6	3	3	3	3	3	3	3	–	–	–	–	–	–	109	Placed with another ♂ on the 32nd day, see entries in Table XII.
4	4	3	5	3	2	–	–	–	–	–	–	–	–	–	–	–	81	Placed with another ♂ on the 28th day, see entries in Table XII.
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	25	Died on the 11th day.
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	66	,, ,, 20th ,,
6	6	5	4	4	6	–	4	4	4	5	3	2	5	–	2	–	138	Placed with ♂ No. 4 on the 36th day, see entries below.
–	4	–	4	5	–	–	–	–	–	–	–	–	–	–	–	–	107	Died on the 26th day.
5	4	3	2	4	–	3	–	7	–	–	–	–	–	–	–	–	101	Placed with ♂ No. 4 on the 30th day, see entries below.
5	6	–	4	6	5	–	–	6	3	–	–	–	–	–	–	–	102	,, ,, ,, 31st ,, ,, ,,
3	4	4	8	7	7	–	–	–	–	–	–	–	–	–	–	–	120	Died on the 29th day.
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	36	Accidentally killed on the 14th day.
3	4	3	–	5	1	6	–	–	–	–	–	–	–	–	–	–	82	Placed with another ♂ on the 28th day } see further
7	4	3	2	3	–	–	–	–	–	–	–	–	–	–	–	–	85	,, ,, ,, 26th ,, } entries in
3	4	7	3	3	3	–	–	–	–	–	–	–	–	–	–	–	92	,, ,, ,, 27th ,, } Table XII.
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	Died on 15th day, presumably malformed as she developed no eggs.
2	6	5	3	1	–	–	–	–	–	–	–	–	–	–	–	–	77	Died on the 25th day.
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	65	Accidentally killed on the 20th day.
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11	Died on the 14th day.
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	31	Died on the 12th day. A stoppage of the oviduct was probably responsible for the death, her body became excessively swollen with eggs which she did not lay.
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	3	Died on the 38th day.
–	–	–	–	–	–	–	–	–	–	–	3	1	2	3	–	–	9	,, ,, 36th ,,
–	–	–	–	–	–	–	–	–	–	–	6	2	–	–	–	–	8	,, ,, 34th ,,

boxes in order to test if the ♀♀ still retained the power to fertilize.

TABLE XI.

Pediculus humanus (vestimenti). *Hatching of eggs at different temperatures.*

A large mass of eggs laid on a strip of cloth was taken from a stock box and divided into three portions, one of which was submitted to each of the following conditions: (*a*) in a room, the air of which was very dry owing to the central heating of the building at 15·6° to 18·4° C., (*b*) in a humid incubator at 24·5° C. constant, (*c*) in a dry air incubator at 36·1° C. constant.

	Day after removal from the stock box on which hatching occurred																							
Temperature	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th	21st	22nd	23rd	24th	Totals
at 15·6° to 18·4° C.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	nil
at 24·5° C.	—	—	—	3	1	—	—	—	—	2	—	—	—	—	4	2	1	1	—	1	—	—	1	—	16
at 36·1° C.	—	—	—	21	32	53	25	12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	143

NOTE. This table only shows the comparative time of hatching, the actual period would have been a few days longer. Prior to the removal of eggs from the stock box it would have contained some in an advanced stage of development. Probably the eggs on the verge of hatching would in some cases be killed or delayed by the sudden alteration in the conditions as the stage immediately preceding hatching seems to be a critical one.

TABLE XII.

Fertility of Pediculus capitis. *Particulars of egg laying after a second ♂ was placed with the ♀♀.*

Reference number of ♀	Day counting from maturity of ♀ when the second ♂ was introduced	Date on which the second ♂ was introduced	Date of copulation when observed	Date when the ♂ was removed	Day, counting from maturity of the ♀, on which eggs were laid after the introduction of the second ♂												Total eggs laid per ♀	Remarks
					26th	27th	28th	29th	30th	31st	32nd	33rd	34th	35th	36th	37th		
4	27th	8. II. 16	—	9. II. 16	—	—	—	3	1	—	—	—	—	—	—	—	4	Died 31st day.
3	32nd	9. II. 16	—	10. II. 16	—	—	—	—	—	—	—	2	1	—	—	—	3	,, 34th ,,
13	28th	10. III. 16	—	11. III. 16	—	—	—	2	3	2	2	2	2	—	—	—	13	,, 35th ,,
14	25th	10. III. 16	14. III. 16	17. III. 16	4	4	3	4	3	—	—	—	—	—	—	—	18	,, 31st ,,
15	27th	13. III. 16	15. III. 16	17. III. 16	—	—	—	2	3	5	2	5	3	3	2	—	25	,, 37th ,,

TABLE XIII.

Fertility of Pediculus capitis. *Particulars of the hatching of the eggs.*

NOTE. There is no necessary relation between the lice emerging on any particular day and the eggs laid on any particular date. It was found impracticable to keep the eggs laid on different days separate, they were allowed to accumulate in the box with the laying ♀ until she was transferred to a new box. The zero date from which the number of days has been reckoned is the date on which the ♂ was placed with the ♀.

Reference number of ♀	Date when the ♀ matured	Date when the ♂ was added	Days counting from the date on which the ♂ was placed with the ♀																				Total no. of eggs laid per ♀	Total no. which hatched	Percentage of possible fertile eggs	Remarks
			8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th	21st	22nd	23rd	24th	25th	26th	27th	29th				
1	4. I. 16	17. I 16	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	40	2	25 %	Eggs laid prior to the introduction of a ♂ all failed to develop.
2	5. I. 16	6. I. 16	–	–	–	–	1	–	–	4	2	3	2	4	6	1	–	2	1	2	–	3	–	62	31	50 %	Ability to fertilize eggs retained for at least 7 days.
3	9. I. 16	9. I. 16	–	–	–	–	–	1	1	2	3	1	1	2	1	1	1	1	1	–	–	–	–	109	16	15 %	,, ,, ,, ,, 3 ,,
4	13. I. 16	13. I. 16	–	–	–	–	–	–	1	2	4	1	1	1	5	–	5	7	5	3	4	5	–	81	44	54 %	,, ,, ,, ,, 12 ,,
5	16. I. 16	16. I. 16	–	–	–	–	4	1	3	2	2	3	2	2	2	–	–	–	–	–	–	–	–	25	21	84 %	,, ,, ,, ,, 8 ,,
6	27. I. 16	27. I. 16	–	–	–	–	–	1	4	–	4	6	–	8	4	6	3	3	4	1	–	–	–	66	44	67 %	,, ,, ,, ,, 8 ,,
7	31. I. 16	31. I. 16	–	–	–	–	1	4	3	5	2	4	6	3	5	5	5	3	5	3	3	4	–	138	61	44 %	,, ,, ,, ,, 11 ,,
8	5. II. 16	5. II. 16	–	2	–	1	1	6	3	4	3	5	8	4	4	6	3	3	8	7	–	2	–	107	70	65 %	,, ,, ,, ,, 12 ,,
9	8. II. 16	8. II. 16	–	–	–	–	2	1	6	3	2	5	4	1	6	6	3	5	–	1	–	–	–	101	45	45 %	,, ,, ,, ,, 11 ,,
10	8. II 16	10 II. 16	–	–	–	–	–	4	3	1	6	3	3	2	6	1	3	1	4	1	–	1	–	102	39	38 %	,, ,, ,, ,, 10 ,,
11	11. II. 16	11. II. 16	–	–	–	–	–	2	3	3	2	4	3	5	7	4	1	1	–	–	–	–	–	120	35	29 %	,, ,, ,, ,, 7 ,,
12	12. II. 16	12. II. 16 15. II. 16	–	–	–	–	–	–	–	2	1	4	2	3	4	1	4	–	3	1	–	–	–	36	25	69 %	,, ,, ,, about 7 ,,
13	13. II. 16	14. II. 16	–	–	–	1	2	1	1	2	10	2	2	2	3	3	1	3	2	1	–	–	–	82	36	44 %	,, ,, ,, at least 11 ,,
14	16. II. 16	16. II. 16	–	–	–	–	–	–	1	–	2	1	1	2	2	1	4	2	2	3	1	1	2	85	25	29 %	,, ,, ,, ,, 7 ,,
15	16. II. 16	18. II. 16	–	–	–	3	4	2	2	1	3	5	2	2	4	3	3	4	3	3	1	1	–	92	46	50 %	,, ,, ,, ,, 10 ,,
16	19. II. 16	19. II. 16 25. II. 16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	nil	nil	—	This female was presumably malformed, her ovaries failed to develop though she lived 15 days, fed vigorously and was observed to pair.
17	19. II. 16	21. II. 16	–	–	–	1	4	1	2	3	5	1	1	1	3	1	4	2	2	2	1	1	–	77	35	45 %	Ability to fertilize eggs retained for at least 9 days.
18	23. II. 16	23. II. 16	–	–	–	–	1	2	–	3	5	3	1	1	6	2	3	7	5	2	1	3	1	65	46	71 %	,, ,, ,, ,, 12 ,,
19	26. II. 16	26. II. 16	–	–	–	–	–	3	3	3	1	–	–	–	–	–	–	–	–	–	–	–	–	11	10	91 %	
20	1. III. 16	1. III. 16	–	–	–	–	–	–	1	1	1	–	3	1	–	–	4	–	–	–	–	–	–	31	11	35 %	
7		6. III. 16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	nil		
9		8. III. 16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9	nil		
10		9. III. 16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8	nil		

TABLE XIV.

Fertility of Pediculus capitis. *Particulars of hatching after a second ♂ was placed with the ♀♀.*

Reference no. of ♀	Day, counting from maturity of the ♀, when the second ♂ was introduced	Days counting from the date on which the second ♂ was placed with the ♀						Total eggs laid per ♀	Total of eggs hatched	Percentage fertile	Remarks
		11th	12th	13th	14th	15th	16th				
4	27th	1	—	—	—	—	—	4	1	25 %	died 31st day
3	32nd	1	—	—	—	—	—	3	1	33 %	,, 34th ,.
13	28th	—	—	—	—	—	—	13	nil	—	,, 35th ,,
14	25th	—	—	—	—	—	—	18	nil	—	,, 31st ,,
15	27th	—	1	—	1	3	1	25	6	24 %	,, 37th ,, *

* Although no more eggs hatched the balance had mostly been fertilized, the larvae developed but died just prior to hatching.

TABLE XV.

Pediculus capitis. *Summary of Tables X, XII—XIV.*

Reference number of ♀	Reference number of ♂	Number of days between maturity and pairing	Number of days between maturity and oviposition	Number of eggs laid before the introduction of a ♂	Number of eggs laid, pairing No. 1	Approximate period of fertility in days	Percentage of fertile eggs	Number of eggs laid, pairing No. 2	Percentage of fertile eggs	Total number of eggs laid per ♀	Average number laid each day	Total number of eggs hatched	Percentage of possible fertile eggs which hatched
1	2	no choice	2	32	40	1	25 %	—	—	40	2·5	2	25 %
2	1	doubtful	3	nil	62	at least 7	50 %	—	—	62	3·9	31	50 %
3	1	,,	2	,,	109	,, ,, 3	15 %	3	33 %	112	3·6	17	15 %
4	2	,,	3	,,	81	,, ,, 12	54 %	4	25 %	85	3·3	45	53 %
5	2	same day	3	,,	25	,, ,, 8	84 %	—	—	25	3·1	21	84 %
6	3	doubtful	3	,,	66	,, ,, 8	67 %	—	—	66	4·1	44	67 %
7	3	,,	2	,,	138	,, ,, 11	44 %	3	nil	141	4·0	61	43 %
8	3	,,	2	,,	107	,, ,, 12	65 %	—	—	107	4·7	70	65 %
9	3	same day	2	,,	101	,, ,, 11	45 %	9	nil	110	3·5	45	41 %
10	4	no choice	3	,,	102	,, ,, 10	38 %	8	nil	110	3·8	39	36 %
11	4	1	2	,,	120	,, ,, 7	29 %	—	—	120	4·4	35	29 %
12	4	probably 3	3	,,	36	about 7	69 %	—	—	36	3·0	25	69 %
13	4	no choice	3	,,	82	at least 11	44 %	13	nil	95	3·1	36	38 %
14	4	doubtful	3	,,	85	,, ,, 7	29 %	18	nil	103	3·8	25	24 %
15	4	,,	2	3	92	,, ,, 10	50 %	25	24 %	117	3·7	52	44 %
16	4	1	...	nil	nil	...	...	—	—	nil	nil	nil	nil
17	4	doubtful	4	,,	77	at least 9	45 %	—	—	77	3·7	35	45 %
18	4	,,	3	,,	65	,, ,, 12	71 %	—	—	65	3·8	46	71 %
19	4	,,	2	,,	11	3	91 %	—	—	11	2·2	10	91 %
20	4	,,	2	,,	31	at least 6	35 %	—	—	31	3·1	11	35 %

Longest ♂ life 30 days. Longest ♀ life 38 days. Average ♀ life 27 days. Number of ♀♀ fertilized by one ♂ 10. Eggs laid by unfertilized ♀♀ did not hatch. The period of fertility lasts about 7 to 12 days. The greatest number of eggs fertilized by a ♀ after separation from a ♂ was 70 (♀ No. 8). The egg laying record in the above experiment ranges between 25 and 141 with an average of 88 and a daily average of 3·7. It is probable that owing to the unnatural conditions of the experiment that these figures are too low as an estimate of the egg production of wild lice.

the real period of fertility exceeded that calculated, it being improbable that there would be no failures among the early laid eggs. As soon as the ♀♀ ceased to lay fertile eggs some of them were given the opportunity of a second pairing. Particulars of the egg laying subsequent to the introduction of a second ♂ are set out in Table II, and of the hatching of the eggs in Table V. It will be seen that a number of the ♀♀ regained fertility after a second pairing, while two were afforded the option of a third. One of these (No. 16) apparently missed the second opportunity, but availed herself of the third and laid a few fertile eggs; the other (No. 18) survived long enough to lay a few more eggs, but none of them hatched. Details are given in Tables III and VI[1].

Although the ♀♀ are stated to have no receptaculum seminis it will be seen that they are able to lay fertile eggs as long as 20 days after the removal of the ♂. This does not compare so unfavourably with fleas, in which the receptaculum is well developed. The ♀ of *Pulex irritans* requires to pair several times if all the eggs she is capable of laying are to be fertilized.

Table VII summarises the previous ones.

Table VIII shows the results of an experiment in differential feeding. Owing to the escape of a ♀ from Box A, it was necessary to remove one from Box B, and to discard observations made prior to this reduction in numbers. Consequently, the experiment covers the later, instead of the earlier, two-thirds of the insects' lives, as was intended. It is probably owing to this that there is a marked falling off in the average number of eggs laid during the reversed order of feeding, when the lice in Box B were fed twice a day and those in Box A only once. Nevertheless, the result shows clearly how dependent egg laying is on food supply—the fertility of the eggs remaining unaffected.

Hatching of eggs. Table IX shows details of the hatching of a number of eggs laid by 10 pairs of *P. humanus* from the date of their maturity until the 30th day. The eggs were placed in an incubator at a constant temperature of 31° C., the air being kept humid by a pan of water with slips of filter paper dipping into the water and hanging over the side of the pans. The high percentage hatching suggests that the conditions were not unfavourable. The variability shown in hatching may have been somewhat enhanced by the fact that the time of examination

[1] It is of course obvious that the percentage of eggs hatching in these Tables, as well as those in the parallel series for *P. capitis* Tables X and XII, has no real relation to the natural fertility of the eggs in the correct sense of the term; the figures are only inserted as a convenience.

occasionally varied by three or four hours. It would be possible for the three records of hatching on the 11th day to be due to an earlier examination than usual on the previous day.

Table XI gives the record of an experiment of the hatching of batches of eggs taken from a stock box and placed under varied conditions. None of the eggs hatched at room temperature, 15·6° to 18·4° C., but a few lice emerged from eggs kept at 24·5° C.

The widely distributed dates of hatching, in some instances after a long interval of time, of these eggs is in marked contrast to the uniformly rapid hatching of eggs kept at 36·1° C. and reminds us of the instance recorded by Warburton (1909), although the period of delay is far shorter.

Although the eggs were not counted, there is no doubt that many died when maintained at 24·5° C.; the batch submitted to cool conditions, 15·6° to 18·4° C., was smaller than the one placed at 36·1° C. but the number must have been upwards of 100.

Pediculus capitis. The attempt with this species to parallel the series of breeding experiments carried out with *P. humanus* were less successful. Possibly the former constituted a chance observation that it would be difficult to repeat without numerous failures, but more probably the conditions were less favourable for the head than for the body louse. There is, however, probably a real difference in the length of life and fecundity of the two insects even if this appears somewhat exaggerated in the protocol.

From Table X it will be seen that four ♂♂ were used in fertilizing the series of 20 ♀♀, the first three ♂♂ dying early in their career, but the fourth fertilized 10 ♀♀ and certainly paired once at least with a malformed ♀ that developed no eggs. A shortage in the supply of virgin ♀♀ was responsible for the fewer opportunities afforded to this ♂ than to the *P. humanus*. The fact that the ♂♂ in many instances were left longer with the ♀♀ than the one day which was usual in the parallel experiment with *P. humanus* probably accounts for there being no failures in fertilization. Similarly the periods during which the ♀♀ retain the power to lay fertile eggs may have been understated since they have been reckoned from the date upon which the male was removed.

Table XIII shows details regarding the hatching of the eggs recorded in Table X. Owing to the earlier loss of the power to lay fertile eggs in this species, the period is less clearly defined as regards its upper limit than in the case of *P. humanus*, but there is little doubt but that it is really shorter by about one-third.

Tables XII and XIV show the distribution in time of egg laying and hatching for the ♀♀ of the series which were afforded the opportunity of pairing with a second ♂.

Although the evidence obtained with regard to *P. capitis* is not so full as for *P. humanus*, it is sufficient to show that the habits of pairing, egg laying and fertilization are similar in both species.

Hybridization.

An attempt was made to hybridize the two insects with a view to obtaining evidence bearing upon the debated question of their right to specific rank. It was found that when single pairs were confined in the same box, the ♂♂ of *capitis* and ♀♀ of *humanus*, or vice versa paired freely. In half the attempts, however, the ♀♀ of *P. capitis* were killed in the act of pairing or died within a day or two, presumably as the result of renewed attempts. It was found necessary, if any number of eggs was required, to remove the ♂ *P. humanus* after the pairing had been consummated. No such precaution was needed in the case of the reverse pairing, and although some of the ♂♂ of *P. capitis* used died early, they succeeded in fertilizing the eggs.

A noticeable feature of the pairing between *P. capitis* ♂ and *P. humanus* ♀ was the disparity of the sexes in the F. 1 generation of some of the crosses. The first trial gave 71 ♂♂ (= 74 %) against 25 ♀♀ (= 26 %), three specimens being killed in the nymph stage. There was considerable mortality in the egg state, which possibly accounted for the small number of ♀♀, although it affords no satisfactory explanation of the disparity of the sexes. No deaths were observed in the larval or nymph stage.

Pairing No. 2 of this cross gave 130 ♂♂ (= 86 %) and 22 ♀♀ (= 14 %), 44 of the ♂♂ had matured before the first ♀♀ developed.

Pairing No. 3 gave 51 ♂♂ (= 51 %) and 49 ♀♀ (= 49 %).

Pairing No. 4 gave 76 ♂♂ (= 68 %) and 35 ♀♀ (= 32 %).

In the F. 2 and F. 3 generations arising from cross pairing No. 1 the number of ♂♂ and ♀♀ appeared normal in all the boxes whether they contained single pairs or the eggs resulting from a number of individuals laid in a stock box. A considerable number of specimens were preserved without selection and an examination of these insects shows the following result:

F. 2 generation, 211 ♂♂ (= 54 %) and 181 ♀♀ (= 46 %).

F. 3 generation, 93 ♂♂ (= 46 %) and 110 ♀♀ (= 54 %) also 80 nymphs.

With the F. 1 generation of reverse cross, *P. humanus* ♂ and *P. capitis* ♀, the disparity was not so obvious.

The first trial failed owing to the death of the ♀ whilst pairing.

A second attempt was more successful and about 50 eggs were laid, the ♀ dying on the 17th day.

The F. 1 generation consisted of 27 ♂♂ (= 55 %) and 22 ♀♀ (= 45 %).

In the third trial the ♀ died after laying one egg.

A fourth attempt was made; two ♀♀ of *P. capitis* being confined with one ♂ of *P. humanus*. One of the ♀♀ died within a day or two and the other was left with the ♂, she laid about 30 eggs, but died within 10 or 12 days.

The F. 1 generation from this pairing consisted of 17 ♂♂ (= 71 %) and 7 ♀♀ (= 29 %).

One or two other attempts were made which resulted in the death of the ♀♀.

Of the subsequent generations reared from the F. 1 generation of the second pairing, a large number of specimens were reared and killed as they reached maturity. They consist of a portion, not the whole, of the offspring of two single pairings and a number of pairs left together in a stock box.

The specimens of the F. 2 generation are

143 ♂♂ (= 57 %), 109 ♀♀ (= 43 %) and 6 nymphs.

The specimens present of the F. 3 generation are

63 ♂♂ (= 50 %), 64 ♀♀ (= 50 %).

In size the specimens of the F. 1 and F. 2 generations of both crosses are generally intermediate between those of *P. humanus* and *P. capitis*, but in the F. 3 generation considerable disparity was noticeable—some very large ♂♂ and some very small ♀♀ being observed.

In the F. 2 generation, observations were made in regard to the egg laying habit, both hair (human) and cloth having been placed in boxes with a few selected pairs of hybrids of the F. 2 generation of the cross *P. capitis* ♂ and *P. humanus* ♀. I noticed that the ♀♀ laid on hair for choice, only a few eggs being attached to the cloth, but the lice showed the instinct to cluster their eggs, a few only being generally scattered along the hairs as is the case with eggs of *P. capitis*[1].

[1] The F. 1 generation of this cross, resulting from a later pairing, show a marked preference for laying on cloth; out of four pairings three ♀♀ laid their eggs on cloth, while one selected hair and laid nearly all her eggs upon it. Unfortunately I was too busy to note the egg laying habit of the F. 1 generation of the earlier pairing.

The ♀♀ of the F. 2 generation of the cross, *P. humanus* ♂ and *P. capitis* ♀, laid their eggs on both cloth and hair, but they showed a preference for laying on hair; clustering was not so noticeable a feature as in the case of the ♀♀ of the reverse cross.

An examination of the eggs laid by these hybrids showed that on the whole they were clearly intermediate in size between those of the species. The eggs are, however, variable in size, both as regards those of the species and those of the hybrids, so that it was easy to match eggs of either *capitis* or *humanus* from the hybrid batch.

No signs of unhealthiness, mortality in moulting or shortening of life in the hybrid insects was observed; the usual life was 30 to 40 days. One pair of the F. 1 generation of the *P. capitis* ♂ and *P. humanus* ♀ cross lived 45 days and their fecundity appeared to be on a par with that of *P. humanus*. They throve just as well under the artificial conditions of rearing as did this species. Although *P. capitis* can be successfully reared in the boxes, the colonies do not show the same rapid and vigorous growth.

There was no noticeable increase of mortality in the eggs laid by the hybrid insects.

No attempts were made to carry the hybrid races beyond the F. 3 generation; they were then in all respects healthy and fertile, probably they could be continued indefinitely. The variability in size referred to above may, however, have been the beginning of a segregating process which would eventually result in the hybrid races being forced back to the specific norm.

Summary.

General comparative note on the two species.

Pediculus humanus (*vestimenti*) is a larger, more robust and less active insect than *P. capitis*,—the ♀♀ having a relatively greater egg-carrying capacity than those of the head louse. The eggs are larger and the number laid (under the conditions of these experiments) is greater, while the habits associated with egg laying differ, although placing the ♀♀ of *humanus* under conditions applicable to *capitis* or vice versa may induce a considerable degree of uniformity. Cross pairings between the insects are easily brought about and the offspring are fertile *inter se*. Hybrid strains were maintained until the F. 3 generation and there seemed no reason, judging from breeding results, why such strains should not be continued indefinitely. Nevertheless

the marked disparity in the sexes of the F. 1 generation of some of the crosses between *P. capitis* ♂ and *P. humanus* ♀ suggests that the parents are specifically distinct.

No such obvious disparity occurred between the sexes of the F. 2 and F. 3 hybrid generation, or of either of the pure stocks[1].

Habits. The body louse exhibits some of the habits of a gregarious animal especially during the moulting phases, also a preference for returning to the same spot for oviposition, which leads to the clustering of its eggs. These habits are shown, though in a less marked degree, by *P. capitis*, and it is possible therefore that they are to some extent the outcome of confinement. Pairing within both species took place at any time during day or night, and was very frequently observed after feeding. ♂♂ with but little food in their alimentary tract were, however, often seen in coitus. The period during which the insects remained paired was frequently observed to be over an hour, but no upper limit was defined.

A ♂ of *P. humanus* fertilized 18 out of 21 ♀♀ placed with him in succession. Four attempts with *P. capitis* were less successful; one ♂ fertilized ten ♀♀ and very possibly might have equalled the *P. humanus* record but for a scarcity of virgin ♀♀ while the experiment was in progress. The longest period during which a ♀ of *P. humanus* retained the power to lay fertile eggs in the absence of a ♂ was 20 days, usually it would seem to be from 16 to 18 days. In the case of *P. capitis* the period was shorter; 12 days being the longest ascertained period, while it was more usually from seven to eleven days.

The greatest number of eggs laid by any one ♀ of *P. humanus* was 295, an average of 6·4 per day—the daily average of a number of ♀♀ being 5·1. *P. capitis* ♀♀ showed a lower fecundity, the highest record being 141 with a daily average of 4—the general average being 3·7. These figures are probably exceeded under natural conditions. An experiment in differential feeding with *P. humanus* (Table VIII) shows clearly that fecundity is dependent on feeding. When extra feeding time over and above seven hours per day was given the average for four ♀♀ was eight per day. It is reasonable to suppose that the average for *P. capitis* would also be increased by unrestricted feeding.

[1] Since the above went to press I have reared two broods of *P. humanus* from crosses between pale and dark forms of this species with a view to discovering if the melanic race shows Mendelian inheritance. In the case of a pairing between a pale ♂ and a dark ♀ there resulted 15 ♂♂ and 54 ♀♀, while from the reverse cross 102 ♂♂ and only 15 ♀♀ were bred. This result qualifies the above suggestion and necessitates further breeding experiments which are now in progress. (See p. 259, paper by Hindle.)

The fertility of the eggs laid was not affected by increased feeding. The greatest number of fertilized eggs laid by a ♀ *P. humanus* after the removal of the ♂ was 115 (♀ No. 9), with a ♀ showing a higher daily laying average this might well be exceeded. With *P. capitis* the parallel figure is 70 (♀ No. 9). The ♀♀ of both species, after arriving at maturity, started oviposition irrespective of their having paired or not, but eggs laid by virgin ♀♀ were invariably infertile.

Length of life. The life of the ♂ *P. humanus* used in the experiment recorded in Table I was 32 days; the longest ♀ life was 46 days, with an average of 34. For *P. capitis* the figures were: ♂ life 30 days; ♀ life 38 days, with an average of 27 days. Whether or not the average lives of the insects would be extended by unrestricted feeding is an open question.

The life of the hybrid insects was not noticeably shorter than that usual for *P. humanus*, and they seemed to thrive better than *P. capitis*.

Tests made with *unfed P. humanus* showed that the longest lives were at a medium temperature of 16° to 18° C., many of the insects living from three to four days, while two lived five and one lived seven days. At 24·5° C. all died within five days. At 36·1° C. all died within three days.

Newly-hatched larvae, unless fed, lived less than 24 hours at 36·1° C., and when kept in a box in the vest pocket they lived but little more than a day; none survived a second day.

Adults kept in a box in the side pocket of a coat lived five days without food; this was in March.

Moulting. 40 young lice were reared in a box carried in a vest pocket and particulars of their moulting recorded.

1st moult: 3 % moulted on the 3rd day; 42 % on the 4th and 55 % on the 5th day.

2nd moult: 15 % moulted on the 7th day; 72 % on the 8th and 13 % on the 9th day.

3rd moult: 5 % moulted on the 10th day; 3 % on the 11th, 55 % on the 12th, 32 % on the 13th day, while 5 % took 14 days to reach maturity.

The ♂♂ usually mature rather earlier than the ♀♀.

Cold. Active specimens of *P. humanus* survived two days at a temperature of − 2·3° C. to − 1·1° C., but none recovered after exposure to these conditions for a week.

Hatching of eggs. Table IX shows that under humid conditions at 31° C. 3 % of the 1300 eggs tested hatched on the 7th day; 56 % on

the 8th; 33 % on the 9th; 8 % on the 10th and ·2 % later on the same day or on the 11th.

A test of batches of eggs taken from a stock box, some of which must have been laid several days previously, showed that none hatched at 15·6°–18·4° C., while at 24·5° C. there was considerable egg mortality, and the hatching period was spread over a longer period than usual, though not to the extent mentioned by Warburton (1909); at 36·1° C. hatching was spread over five days and the mortality was not excessive.

To give some idea of the possible rate of multiplication of *P. humanus* we may estimate the egg period as 12 days and a further 12 days to the maturity of the ♀♀. Allowing an average of eight eggs per day, spread over a fertility period of 40 days, we find that, during her life, a single ♀ may have 4160 offspring.

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NOTES ON THE BIOLOGY OF *PEDICULUS HUMANUS*.

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With a Foreword by GEO. H. F. NUTTALL, F.R.S.

(*From the Quick Laboratory, University of Cambridge.*)

Foreword.

During the two years preceding the outbreak of war, Dr Hindle was engaged in researches on the biology of lice. Soon after the war began he joined the army, leaving his notes with me. He has now been in France for over a year and is serving as Captain in the Royal Engineers. An abstract of his work has been set up in type for many months for publication by the Local Government Board but his contribution has been withheld unavoidably through a desire that it should be supplemented by further work which has since been conducted in the Quick Laboratory.

In view of Mr Bacot's interesting results described in the foregoing paper, it seems advisable to publish some of Dr Hindle's ms. notes which deal with problems not possessing medical interest, viz. the occurrence of ♂ and ♀ broods and a reference to an extended series of experiments he made upon the inheritance of melanism in *Pediculus humanus*, subjects referred to by Mr Bacot on p. 256 (see also his footnote). As Dr Hindle states, his results are inconclusive, but they are decidedly of interest, and, as he was the first in the field, credit should be given to him for what he has done. He hopes in the future to continue his investigations.

For several years we have raised *P. humanus* without difficulty by feeding them twice daily upon the human subject and keeping them,

during the intervals, in a thermostat, preferably at 30° C. We have also raised them through their life-cycle on man making detailed observations on their biology. A full account of our investigations is in preparation for the press.

G. H. F. N.

On the Inheritance of Sex.

The temperature of 30° C. was found to be the most satisfactory for rearing lice, and by feeding the insects twice a day large numbers were raised and maintained in the laboratory through five generations after which the experiment was stopped owing to the interruption caused by the war. In every case pairs of lice were isolated in glass tubes and kept separate so that the offspring of each pair could be raised separately. The results have been of great interest for they shew that in the louse the inheritance of sex is of a very peculiar type. The broods may be either of the following characters:

(*a*) Entirely male.
(*b*) ,, female.
(*c*) Male and female with the number of males predominating.
(*d*) Male and female with the number of females predominating.

Setting aside the various colour variations that were obtained the composition of some of the broods is shown below.

First Series.

Exp. 1. *Broods from lice of unknown parentage.*

(*a*) Darkly pigmented ♀ × white ♂; only 18 eggs were laid of which 9 were reared to the adults. (Male brood.)	9 ♂♂
(*a* 1) The above ♂ was crossed with another ♀; 17 eggs were laid but only 3 were reared to adult stage. (Mixed brood.)	2 ♂♂ 1 ♀
(*b*) Darkly pigmented ♂ × white ♀; 36 eggs laid, of which 17 hatched and 12 became adult. (Male brood.)	12 ♂♂
(*c*) Dark ♂ × dark ♀; 23 eggs were laid of which 20 hatched and 15 became adult. (Mixed brood.)	10 ♂♂ 5 ♀♀
(*d*) Dark ♂ × dark ♀; 60 eggs were laid from which 35 nymphs hatched and 26 became adult. (Male brood.)	26 ♂♂

(*e*) Dark ♂ × white ♀; 34 eggs were laid of which 5 hatched and 4 became adult. (Female brood.) 4 ♀♀

(*f*) White ♂ × white ♀; 63 eggs were laid of which 48 hatched and 32 became adult. (Female brood.) 32 ♀♀

(*g*) White ♂ × white ♀; 51 eggs laid of which 29 hatched and 25 became adult. (Mixed brood.) 17 ♂♂, 8 ♀♀

In the above series of experiments it will be noticed that three of the broods were entirely male; two, female; whilst three were mixed broods in which the number of males was approximately double the number of females. The results of experiments (*a*) and (*a* 1) show that the same male can produce both male and mixed broods.

EXP. 2. *Broods from lice of known parentage*, taken from the above-mentioned reared broods. The nature of the brood the parent came from is indicated in brackets, as also the number of the experiment[1].

(*a*) ♂ (*c* ♂♀) × ♀ (*c* ♂♀). 17 adults were reared. (Mixed brood.) 14 ♂♂, 3 ♀♀

(*b*) ♂ (*b* ♂♂) × ♀ (*c* ♂♀). 34 adults were reared. (Female brood.) 34 ♀♀

(*c*) ♂ (*b* ♂♂) × ♀ (*c* ♂♀). 48 adults were reared. (Female brood.) 48 ♀♀

In this series only three broods were reared and an accident to the incubator prevented further experiments with this series.

SECOND SERIES (17. I. 14)

First Generations. Broods from lice of unknown parentage[2].

(1) Medium ♂ × white ♀; 45 nymphs hatched of which 27 became adult. Male brood. 27 ♂♂

(2) White ♂ × white ♀; 58 nymphs hatched, of which 45 became adult. Female brood. 45 ♀♀

(3) White ♂ × white ♀; 38 nymphs hatched, of which 32 became adult. Female brood (?). 31 ♀♀, 1 ♂(?)

(4) White ♂ × medium ♀; 16 nymphs hatched, of which 9 became adult. Female brood. 9 ♀♀

[1] For example, ♀ (*c* ♂♀) × ♂ (*b* ♂♂) signifies that the female came from the mixed brood of (*c*) in experiment 1, and was crossed with a male from the male brood (*b*) of the above series.

[2] The terms dark, medium and white are used to indicate roughly the pigmentation of the lice. Details of the colouration of broods will be given later.

(8) Medium ♂ × medium ♀; 42 nymphs hatched, of which 31 became adult. Mixed brood. — 18 ♀♀, 13 ♂♂

(9) White ♂ × white ♀; 9 nymphs hatched, of which two became adult. Female brood. — 2 ♀♀

(11) Medium ♂ × medium light ♀; 39 nymphs hatched, of which 30 became adult. Male brood (? mixed). — 28 ♂♂, 2 ♀♀(?)

(12) Medium ♂ × medium ♀; 19 nymphs hatched, of which 11 became adult. Mixed brood (?). — 9 ♀♀, 2 ♂♂

(13) Medium ♂ × medium light ♀; 46 nymphs hatched, of which 38 became adult. Male brood. — 38 ♂♂

(15) Dark ♂ × dark ♀; 9 nymphs hatched, of which 6 became adult. Female brood. — 6 ♀♀

(15 *a*) Dark ♂ (from 15) × dark ♀; 50 nymphs hatched, of which 24 became adult. Female brood. — 24 ♀♀

(16) Medium ♂ × medium ♀; 66 nymphs hatched, of which 64 became adult. Female brood. — 64 ♀♀

(17) Medium ♂ × white ♀; 42 nymphs hatched, of which 29 became adult. Female brood (?). — 27 ♀♀, 2 ♂♂ (?)

(18) White ♂ × white ♀; 25 nymphs hatched, of which 10 became adult. Mixed brood (?). — 8 ♂♂, 2 ♀♀

(19) White ♂ × white ♀; 39 nymphs hatched, of which 26 became adult. Female brood. — 26 ♀♀

In this first generation, therefore, there are seven female broods, two male broods and one mixed brood. In addition there are five doubtful broods, three possibly female and two male, in which there are one or two examples of the other sex occurring. It is probable that in these cases individuals had managed to come in from another brood, for when feeding two or three broods at the same time, it was difficult to prevent them wandering.

Second Generation. Broods from lice taken from the first generation. The numbers refer to the above-described experiments of the first generation.

(1) 18 ♂ (? ♀♂) × 19 ♀ (♀♀); 33 nymphs hatched, of which 2 became adult. Male brood. — 2 ♂♂

(2) 8 ♂ (♀♂) × 8 ♀ (♀♂); 12 nymphs hatched, of which 5 became adult. Mixed brood. — 4 ♀♀, 1 ♂

(4) 13 ♂ (♂♂) × 15*a* ♀ (♀♀); 45 nymphs hatched, of which 28 became adult. Mixed brood. — 15 ♀♀, 13 ♂♂

(6) 13 ♂ (♂♂) × 3 ♀ (♀♀); 20 nymphs hatched, of which 16 became adult. Mixed brood. — 13 ♂♂, 3 ♀♀

(8) 18 ♂ (♀♂) × 8 ♀ (♀♂); 12 nymphs hatched, of which 7 became adult. Mixed brood. { 4 ♂♂ 3 ♀♀

(10) 13 ♂ (♂♂) × 8 ♀ (♀♂); 16 nymphs hatched, of which 7 became adult. Mixed brood. { 5 ♂♂ 2 ♀♀

(11) 13 ♂ (♂♂) × 19 ♀ (♀♀); 27 nymphs hatched, of which 6 became adult. Mixed brood. { 3 ♂♂ 3 ♀♀

(12) 13 ♂ (♂♂) × 19 ♀ (♀♀); 19 nymphs hatched, of which 2 became adult. Female brood. 2 ♀♀

(13) 18 ♂ (♀♂) × 19 ♀ (♀♀); 19 nymphs hatched, of which 1 became adult. Female brood (?). 1 ♀

In this second generation, there are apparently six mixed broods, two possibly male and female and the last one is uncertain. In any case the numbers of the adults are so small that it is impossible to say whether these three broods were either mixed, or would have been respectively male and female.

Third Generation. Broods from lice taken from the second generation. The numbers refer to the number of the experiment in that generation.

(2) 6 ♂ (♂♂) × 4 ♀ (♀♀); 78 nymphs hatched, of which 54 became adult. Male brood. 54 ♂♂

(3) 6 ♂ (♂♂) × 4 ♀ (♀♀); 39 nymphs hatched, of which 28 became adult. Male brood. 28 ♂♂

(4) 6 ♂ (♂♂) × 10 ♀ (♂♀); 19 nymphs hatched, of which 12 became adult. Mixed brood. { 6 ♂♂ 6 ♀♀

(5) 6 ♂ (♂♂) × 4 ♀ (♀♀); 56 nymphs hatched, of which 42 became adult. Female brood. 42 ♀♀

(6) 6 ♂ (♂♂) × 8 ♀ (♂♀); 31 nymphs hatched, of which 20 became adult. Mixed brood. { 12 ♂♂ 8 ♀♀

(7) 6 ♂ (♂♂) × 10 ♀ (♂♀); 38 nymphs hatched, of which 22 became adult. Mixed brood. { 15 ♂♂ 7 ♀♀

(8) 6 ♂ (♂♂) × 2 ♀ (♂♀); 30 nymphs hatched, of which 17 became adult. Mixed brood. { 10 ♂♂ 7 ♀♀

(9) 8 ♂ (♂♀) × 4 ♀ (♀♀); 43 nymphs hatched, of which 32 became adult. Female brood. 32 ♀♀

(10) 8 ♂ (♂♀) × 4 ♀ (♀♀); many eggs were laid but all were sterile.

(13) 10 ♂ (♂♀) × 4 ♀ (♀♀); 47 nymphs hatched, of which 37 became adult. Female brood. 37 ♀♀

(14) 10 ♂ (♂♀) × 4 ♀ (♀♀); 51 nymphs hatched, of which 37 became adult. Female brood. 37 ♀♀

(17) 11 ♂ (♂♀) × 4 ♀ (♀♀); 36 nymphs hatched, of which 13 became adult. Female brood (?). { 12 ♀♀ 1 ♂ (?)

Fourth Generation. Broods from lice taken from the third generation. The numbers refer to the number of the experiment in that generation.

(2) 2 ♂ (♂♂) × 8 ♀ (♂♀); 5 nymphs hatched, of which only 1 became adult. Male brood (?). 1 ♂

(4) 2 ♂ (♂♂) × 5 ♀ (♀♀); 37 nymphs hatched, of which 13 became adult. Mixed brood. 10 ♀♀, 3 ♂♂

(6) 3 ♂ (♂♂) × 13 ♀ (♀♀); 30 nymphs hatched, of which 14 became adult. Male brood. 14 ♂♂

(7) 2 ♂ (♂♂) × 5 ♀ (♀♀); 16 nymphs hatched, of which 3 became adult. Female brood. 3 ♀♀

(8) 2 ♂ (♂♂) × 5 ♀ (♀♀); 36 nymphs hatched, of which 11 became adult. Male brood. 11 ♂♂

(14) 2 ♂ (♂♂) × 7 ♀ (♂♀); 29 nymphs hatched, of which 10 became adult. Male brood (? mixed). 9 ♂♂, 1 ♀

(16) 2 ♂ (♂♂) × 7 ♀ (♂♀); 18 nymphs hatched, of which 3 became adult. Female brood. 3 ♀♀

(17) 2 ♂ (♂♂) × 5 ♀ (♀♀); 24 nymphs hatched, of which 2 became adult. Male brood. 2 ♂♂

Some of the individuals from these experiments were used again for the two following:

(2/6) 2 ♂ (♂♂) (from Exp. 2 of this generation) × 13 ♀ (♀♀) (from Exp. 6 of this generation); 10 nymphs emerged, of which 4 became adult. Mixed brood. 3 ♀♀, 1 ♂

(7/14) 2 ♂ (♂♂) (from Exp. 7 of this generation) × 7 ♀ (♂♀) (from Exp. 14 of this generation); 11 nymphs emerged, of which 5 became adult. Mixed brood. 4 ♂♂, 1 ♀

Fifth Generation. Broods from lice taken from the fourth generation. The numbers refer to the number of the experiment in that generation.

(2) 7/14 ♂ (♂♀) × 7 ♀ (♀♀); 4 nymphs hatched and only one ♀ became adult. 1 ♀

(3) 7/14 ♂ (♂♀) × 7 ♀ (♀♀); 15 nymphs hatched and 4 became adult. Mixed brood. 3 ♀♀, 1 ♂

(13) 6 ♂ (♂♂) × 2/6 ♀ (♂♀); 8 nymphs hatched, and 4 became adult. Female brood. 4 ♀♀

During the last two generations the lice gradually became weaker and only a few eggs were laid, most of which were sterile.

The experiment was discontinued at this stage as the few adults that were left alive were so feeble that they would not have been capable of reproduction.

Observations on Breeding in relation to Melanism.

The various colour varieties were isolated, and attempts made to produce pure strains, the results of which will be given in detail on a future occasion. It will be sufficient to say here that a pure white strain was easily obtained, but great difficulty was experienced in obtaining pure strains of the darker races. There seems no doubt that the method of inheritance is alternative, but whether associated with the sex or not is not yet decided.

Conclusions.

The interruption caused by the war has prevented these experiments being completed and the results obtained are insufficient to afford a reasonable explanation of the peculiar mode of sex inheritance in *Pediculus humanus* which has come to light. It is difficult to explain the results of the experiments in the case of the third generation where apparently the same crosses (♂♂ × ♀♀) have produced pure ♂ broods in two cases and a pure ♀ brood in one case. Whereas pure unpigmented strains were readily raised, difficulty accompanied the attempt to obtain pure darkly pigmented strains. The method of inheritance in respect to pigmentation appears to be alternative. The author hopes to resume his investigations at the conclusion of the war.

THE LIFE HISTORY OF AMOEBAE OF THE *LIMAX* TYPE IN THE HUMAN INTESTINE.

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(With Plate II, and 1 Text-fig.)

At the time investigators were trying to cultivate the amoebae of the human intestine (*Entamoeba coli* and *E. histolytica*), amoebae were often encountered in the cultures which were referred to the species "*Amoeba limax*," with many sub-species. Subsequently it was found that the cysts of these cultural amoebae are very common and that it is possible to cultivate amoebae from nearly every source. Consequently it was concluded that these forms do not really live in the human intestine but that the cultural amoebae developed from cysts, occasionally ingested with food, the cysts not having developed in the intestine. This is Walker's (1911) view, but Chatton and Lalung Bonaire (1912) hold that the limax amoebae (hereafter called limax) can live in the intestine, not only in the form of cysts but also as motile amoebae. Cultures made from these stools showed amoebae and uninucleate cysts. The latter were not found in the faeces, which showed only the motile stages, without however any signs of division. The cultures showed this amoeba to be of the common limax-type with a vesicular nucleus containing a large karyosome. These observations are important because they contradict the hypothesis that *Entamoebae* when cultivated show the features of limax. There is

therefore no reason to suppose that the limax of cultures from stools are derived from *Entamoebae* because limax already pre-exist in faeces. These observations, moreover, explain why limax are found in cultures from the pus of liver abscesses, for this is only conceivable by supposing the limax to be real intestinal parasites, a supposition now proved to be correct. Chatton and Lalung Bonaire's observations were confirmed by Wenyon (1912, 1913); who observed the presence of limax in human faeces over a considerable period of time. These amoebae were not pathogenic for kittens. Mathis (1913 *a*) seldom found limax in human faeces; similar amoebae cultivated from the stools of an ape (1913 *b*) are considered to be derived from cysts passing through the intestine without developing there. Gauducheau (1912, 1913) observed small amoebae of the cultural type, resembling his *Entamoeba phagocytoides* (also a cultural form), he found them in dysenteric stools. Whitmore (1913) made similar observations in Florida, but these amoebae may not have been true intestinal parasites, as the stools were not freshly deposited. James (1915) was the first to observe not only amoebae but also cysts in fresh stools, he saw them continually during a period of three weeks. The cysts were uninucleate and of the common limax type. Job and Hirtzmann (1916) also saw limax in the human intestine, but they regarded them as developing forms of *E. histolytica*.

From these observations there can hardly be any doubt, that limax somctimes occur as true intestinal parasites but it remains to be determined, whether these forms are identical with the cultural limax.

We have observed the intestinal limax (amoeboid stages and cysts) in three cases. In the first case, where only a few observations were made, the limax seemed to be identical with the common cultural type, but in the two others where observation was continued for several months, the morphology of the cysts differed from that of the type commonly found in limax cultures.

The first case is that of a European woman in Sumatra, suffering from chronic diarrhoea. The digestion of amyloid substances was insufficient as appeared by the abundant presence of starch grains and *Bacillus amylobacter* in the stools, especially after ingestion of food containing much amylum (bananas). Besides limax the stools showed the presence of *E. coli* and *Chilomastix mesnili*.

The limax referred to measure 7–8 μ, sometimes showing a distinct ectoplasm and broadly lobose pseudopodia (Pl. II, fig. 3). The entoplasm contains small (figs. 1–2) or larger vacuoles, the nucleus is of the

common limax type showing a dark staining karyosome surrounded by a clear halo, the latter being sometimes enclosed by a nuclear membrane (figs. 1–2). Besides amoebae showing irregular outlines (due to their being fixed when moving about), rounded forms are observed (fig. 4) probably representing initial stages of encystment. Similar forms, measuring 5–6 μ, are seen surrounded by a distinct cyst wall (figs. 5–6). The karyosome of these cysts produces small chromatic granules (fig. 5) which are found afterwards in the cytoplasm. The next stage shows division of the nucleus (fig. 7), the cyst now containing two nuclei and some (commonly two) small chromatic particles. Sometimes the nuclei are dissolved into many chromatic granules (fig. 9) but this is not a common occurrence and is probably to be considered as a sign of degeneration. Stages similar to these represented by figs. 5–6 are described by James, who, however, did not find binucleate cysts. Although most of the cultural limax show only uninucleate cysts, binucleate cysts are also described as *e.g.* by Nägler (1909) in *Amoeba diploidea* and consequently it is probable that the amoeba found in this case, was a common limax form, temporarily developing in the human intestine. We want to emphasize however that in this case the stools were always examined, immediately after deposition; consequently errors resulting from examination of stools where amoebae had time to develop outside the host, are to be excluded.

The second case referred to is that of a Javanese surgeon for the time residing in Holland. There are some records of dysentery in early youth but no definite information was to be obtained and neither *Entamoeba histolytica*, nor *E. coli* or dysentery bacilli were to be found. The patient complained of intermittent pains in the bowels accompanied by fluid or semi-fluid stools containing some mucus. In the stools of normal consistency no parasites were to be seen, but in the semi-fluid stools were found *Blastocystis hominis* and limax (amoebae and cysts). The case was observed regularly from March 25th till July 16th. The following table shows the results:

March 25th. 4-nucleate cysts.

April 2nd. 2- and 4-nucleated cysts, amoeba.

April 8th. *Id.* more amoebae.

April 9th. After ingestion of 25 grammes $MgSO_4$ cysts and many amoebae; after the stools have become quite fluid only amoebae.

Weekly inspection till July 16th. Amoebae and cysts when stools were fluid.

Observations of fresh material. The amoebae move very slowly and die quickly under the cover-glass. This can be demonstrated by examining them in eosin solution. The amoebae are then seen as white patches on a red background, vacuoles being indistinct, the nucleus invisible. When the amoeba dies, the cytoplasm becomes less refringent and is faintly stained by the eosin; the nucleus is stained more deeply, clearly showing the surrounding halo. Often the nucleus

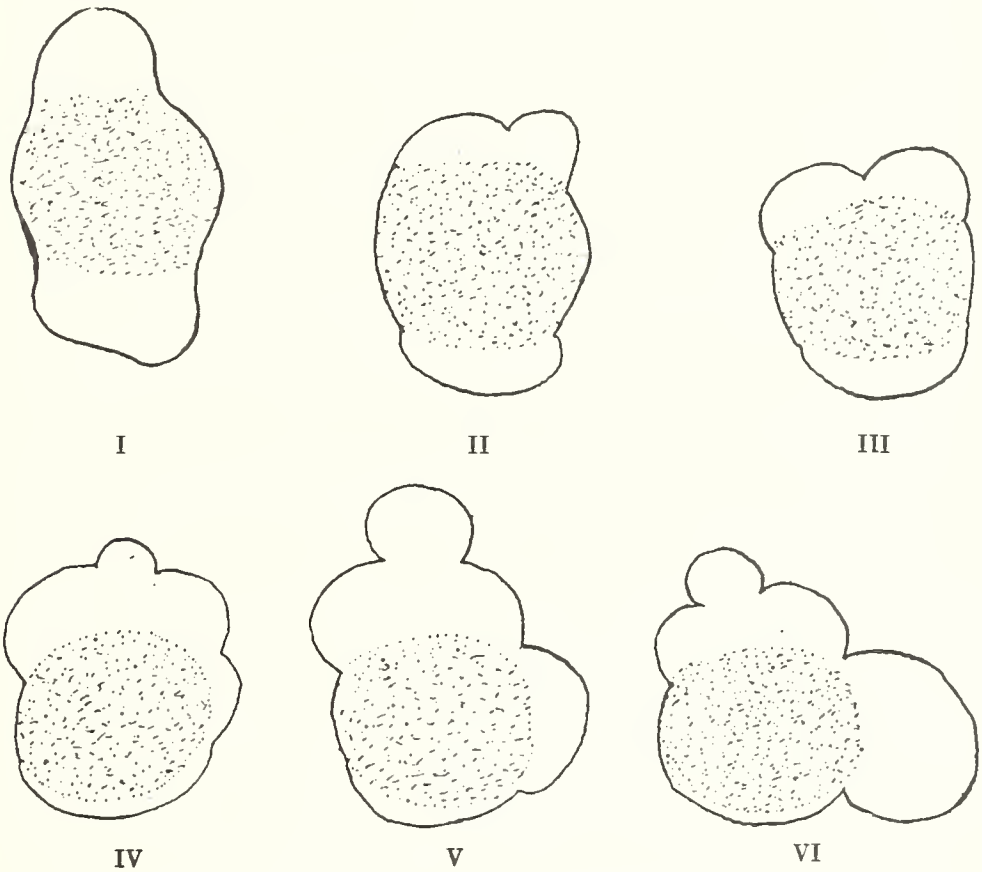

Fig. 1. A limax amoeba from case No. 2, drawn at intervals when moving.

is expelled from the amoeba when dying. The amoebae found in the fluid faeces after purgation with sulphate of magnesia are more actively motile. The pseudopodia are stumpy, ordinarily one at a time is extruded (*cf.* Text-fig. 1). No contractile vacuole was observed. Since the amoebae are easily killed when outside the human body it would seem that they are real parasites (cultural limax remains alive for a much longer time under similar conditions). Moreover after the use of a strong purgative, the amoebae found (they came from some part

of the ileum and not, as the others, from the colon) were not only more motile but much more resistant, which points to their natural habitat not being the colon but the ileum or possibly the jejunum.

The cysts are very resistant outside the host. They remain alive in the faeces for more than a month; and are consequently much more resistant than the cysts of *E. histolytica* which only remain alive for a few days unless they are placed in clear water (*cf.* Kuenen and Swellengrebel, 1913). When treated with Lugol's solution or iodine, some cysts stain yellowish, others stain yellowish with a spherical red-brown mass in the interior. This brown sphere (figs. 34–36) represents a vacuole containing glycogen resembling similar glycogen-vacuoles within the cysts of *E. coli* and *E. histolytica* (as described by Kuenen and Swellengrebel) and of *E. chattoni* and *E. ovis* (Swellengrebel, 1914). These glycogen-vacuoles seem to be of common occurrence in intestinal amoebae. When the cysts are kept alive outside the body the glycogen-vacuoles gradually disappear within a week and at last all the cysts are without a vacuole (4-nucleate cysts).

We did not succeed in cultivating this amoeba from stools containing many motile forms or cysts.

Observation of fixed and stained preparations. Preparations fixed wet in corrosive alcohol and stained with iron haematoxylin showed the following forms:

Amoebae (Pl. II, figs. 10–17). They measure 4–8 μ, sometimes with distinct although narrow ectoplasm (fig. 10). The nucleus of the well-known limax type generally lacks a distinct membrane, sometimes a granule can be made out in the centre of the karyosome (fig. 11). Nuclear division sets in by the karyosome assuming the dumb-bell shape (fig. 12) and subsequently dividing (fig. 13). Afterwards the vesicular part of the nucleus is equally divided, the two parts of the karyosome remaining linked together for some time by a chromatic filament (fig. 14). The disappearance of the latter marks the final stage of the nuclear division (figs. 15, 16). Sometimes amoebae are found showing the karyosome in the form of a crescent or a rodlet, in this form it may divide, the parts being united by linin filaments (fig. 17). Probably these forms should not be considered as normal.

Cysts. The younger stages are uninucleate with a distinct cyst wall and a large karyosome (fig. 18). They correspond to the cysts seen by James. In the next stage the vacuole appears which was shown to contain glycogen (fig. 19), the nucleus divides (fig. 20) and near the nuclei a few chromatic granules make their appearance, each

surrounded by a clear halo and so resembling small nuclei. The cyst now contains a vacuole, 4 large and 1–2 smaller nuclei or 5 nuclei of the same dimension (figs. 22, 23, 25). The cyst wall is especially distinct in cysts showing shrinkage (fig. 25). The vacuolate cysts measure 5–6 μ.

The final stage of encystment is represented by the 4-nucleate cysts without vacuole (figs. 26–32), formed by the gradual disappearance of the vacuole (figs. 23–26). The cytoplasm has a minute alveolar structure, the cyst wall only shows after shrinkage (fig. 28). Commonly these cysts contain 4 small nuclei (figs. 28, 29–32), sometimes the karyosomes are larger and of granular structure (figs. 27, 31). Often smaller additional nuclei are found corresponding to the same formations in the vacuolate cysts (fig. 30). Finally these additional nuclei disappear, but before doing so they often exhibit signs of division (figs. 31, 32).

It would be an easy task to construct a series from these figures showing a complete autogamy:—fig. 19, initial stage of encystment; fig. 20, first nuclear division; fig. 22, 5-nucleate stage; fig. 21, three nuclei (reduction nuclei) degenerate, two of them having divided beforehand, the remaining two (sexual nuclei) about to copulate; fig. 18, fusion of the sexual nuclei, formation of the synkaryon; fig. 24, first division of synkaryon; fig. 29, second division of the same; fig. 30–32, further division of reduction nuclei before disappearing.—But we prefer simply to state that the cysts initially containing a glycogen vacuole become afterwards avacuolate and that the uninucleate amoeba ultimately produces a 4-nucleate cyst with a 5- or 6-nucleate intermediate stage; some of the nuclei (probably the small ones, judging by fig. 30) must disappear. Undoubtedly this interpretation is not so interesting as that including the autogamy, but we think it the best, even if it should be proved afterwards that autogamy really occurs in this species. We do not want to follow the custom adopted by some authors of describing as reduction nuclei all sorts of chromatic granules observed during a process of encystment (in which the nuclear changes are somewhat intricate) and we are disinclined to consider the whole process as an example of autogamy.

The 4-nucleate cysts containing no vacuoles are somewhat larger than the vacuolate cysts (6–6·5 μ). This difference probably depends upon the latter being more liable to shrinkage during fixation (the vacuole containing much water) than the former.

Forms were frequently noted (fig. 33), resembling 4-nucleate cysts but being much larger (8 μ) and without a trace of a cyst wall. Possibly

these forms have left the cyst and are about to return to the motile stage.

In the third case, an amoeba was found in the stools of a European who had resided for some years in the Dutch Indies. This amoeba resembled the former one in all details. It was found in company with *E. coli* and *Blastocystis hominis*. No pathogenic action was observed.

Conclusions.

One of the amoebae herein described may be an ordinary limax form. The other is of the limax type but the cysts are different and by its physiological characters it is to be considered a true intestinal parasite. It is uncertain whether this amoeba is pathogenic or not. In the second case the intestinal complaint could only be ascribed to it, no other parasites being present, but in the third case the carrier of the parasite was perfectly healthy. The morphological features of the parasite permit of its being distinguished without difficulty from other intestinal parasites: the cysts and motile forms of *Entamoeba coli* and *E. histolytica* are larger; the 4-nucleate cysts of *Chilomastix mesnili*, although of the same size, show different nuclear (vesicular) structure. *Blastocystis* is often of the same size and its nucleus is of a similar structure, but its characteristic highly refractile character should prevent confusion.

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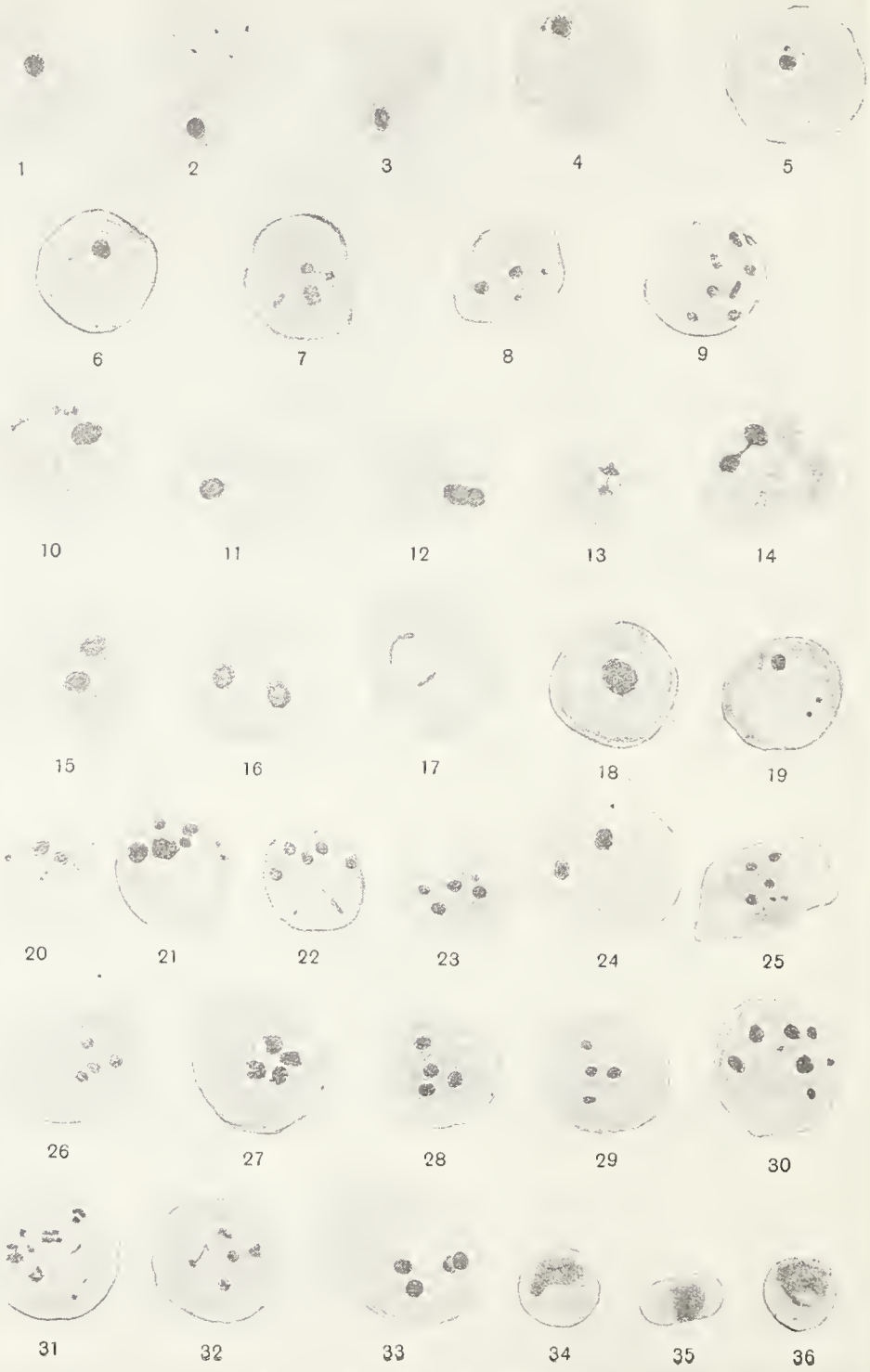
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EXPLANATION OF PLATE II.

Figs. 1–9. Amoebae and cysts from case No. 1.

Figs. 1–3. Amoeboid stages.
Fig. 4. Rounded form.
Fig. 5. Uninucleate cyst. Extrusion of chromatic granule from the nucleus.
Fig. 6. Uninucleate cyst.
Figs. 7–8. Binucleate cyst with two additional nuclei.
Fig. 9. Cyst with scattered chromatin.

Figs. 10–36. Amoebae and cysts from case No. 2.

Figs. 10–11. Amoebae, fig. 11 showing granule in the karyosome.
Figs. 12–16. Division of the nucleus.
Fig. 17. Abnormal features of the karyosome.
Fig. 18. Initial stage of encystment.
Figs. 19–22. Vacuolate cyst.
Fig. 25. *Idem*. showing cyst wall.
Figs. 23, 30–32. Avacuolate cyst with additional nuclei.
Fig. 24. Avacuolate cyst with only two nuclei.
Figs. 26–29. 4-nucleate cysts.
Fig. 33. 4-nucleate large form without cyst wall.
Figs. 34–36. Cysts treated with Lugol's solution showing glycogen vacuole. (Lower magnification than the other figures.)

URETHRAL SPIROCHAETOSIS.

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(With 4 Text-figures and 1 Chart.)

Spirochaetes have been found in a large number of human diseases, in the blood, in various parts of the intestinal tract from the mouth to the rectum, in sores and ulcers, and in numerous gangrenous, phagedaenic, and inflammatory conditions; but so far as I am able to ascertain they have not previously been found in the urethra. Recently a case of acute urethritis has come under my notice in which a spirochaete was found which appeared to be the specific cause of the disease, and I believe that an account of this infection may be of interest. I am indebted to Dr A. B. Tighe for bringing this case under my notice, for permitting me to examine it whenever I wished, and for furnishing all the materials necessary for investigation, and I take this opportunity of expressing my sincere thanks to him.

The patient, a steward boy 21 years of age and a native of the Gold Coast, West Africa, was admitted to the Accra hospital on the 29th July, 1916. About a fortnight previously he had been seen as an out-patient by Dr Tighe and had complained of fever, shivering, and pains in his shoulders, symptoms which had troubled him for about a week. His temperature on this occasion was 100° F. He received treatment but did not persevere with it, and being still unwell and unfit for work, was sent back on the date mentioned to be treated in hospital. On admission he looked ill and low-spirited, his temperature was 99° F., he complained of pains in the shoulders and left hip, but he had no symptoms of malaria and there were no swellings of the painful joints. It was discovered, however, that he was suffering from acute urethritis the discharge from which had started only the day before (28th July).

The urethral discharge was profuse, thick, foul smelling and tinged with blood. It resembled the pus from an abscess more closely than

gonorrhoeal discharge, and all recent exposure to gonorrhoeal infection was denied. There was no pain on passing water and no local tenderness was admitted. On examining the discharge it was found to contain innumerable spirochaetes. It is probable that the patient had had gonorrhoea shortly before his illness began, and the joint pains complained of may have been connected with this, but at the time he came under observation he was not suffering from this disease.

The discharge continued to be abundant until the 2nd August when it was only slight and on the following day it had ceased. The discharge, therefore, persisted for six days. The treatment given during this period was two intramuscular injections of $\frac{1}{3}$ gr. perchloride of mercury on 31st July and 1st August respectively[1].

The Movements of the Spirochaetes and other Features observed during Life.

The spirochaetes were very active, moving with great rapidity about the field of the microscope or lashing out in all directions in a relatively restricted area. The movements as is frequently the case could be analysed into components, namely lashing movements, undulatory flexions of the body, and corkscrew or helicoid movements; and in addition Catherine-wheel-like movements were common. In very active organisms the lashing and undulant movements predominated and obscured the helicoid motion to a great extent. In less vigorous spirochaetes the undulations were seen to be most marked at the extremities, the middle portion of the parasite being relatively infrequently flexed. Spirochaetes exhibiting active lashing and undulant movements appeared to have relatively few coils, but as their movements slowed down the number of coils increased so that the quiescent organisms were always seen to have a well coiled body. This was I believe due to the helicoid movement persisting after the lashings and undulatory flexions had ceased, but whatever the true explanation may have been there was no doubt about the fact that the quiescent organisms showed a great number of coils. So far as the undulant waves were concerned, however, it was the most active spirochaetes that showed the greatest number.

[1] In a letter dated 30. ix. 1916, Dr Macfie states "That the patient returned to hospital a month later with severe iritis, and it was feared for some days that he would lose the sight of both eyes, but after an injection of galyl he began to improve rapidly, and, with a continuance of treatment by bichloride of mercury injections, made a complete recovery. This is rather interesting in view of the frequency of iritis in other spirochaetoses."—Ed.

In a recent paper on *S. eurygyrata* Fantham (1916) has stated that "The coil of the helix of a rapidly moving spirochaete is much closer than that of a slowly moving organism." Although this assertion is made in the form of a generalisation it was perhaps intended only to apply to the rotatory movements, for it certainly would be incorrect to apply it without this qualification to the spirochaete found in this case of urethritis. I again and again watched individual spirochaetes from this patient and invariably observed that whereas when the lashing and undulatory movements were most marked, that is when movement was most rapid, the coils of the helix were relatively few, they became more numerous as the organisms became quiescent. When an organism just removed from the body was watched continuously until it was completely immobilised it was seen first to cease to exhibit lashing movements, then to lose its undulatory movements, and finally to give up rotation. The rotatory movements became slower and slower as time went on, but even when they had entirely ceased and the organism was presumbly moribund the body was still well coiled. As a rule there did not seem to be any sensible difference in the closeness of the spiral in the rapidly rotating organisms and in those revolving more slowly or absolutely at rest. It is true, however, that some of the spirochaetes when spinning round their long axes at a very great rate, as they sometimes did, appeared to become more closely coiled; but even this might have been an optical effect, and it would in any case be incorrect to say that the number of coils was an index of the rate of motion since the most active individuals were those showing lashing movements which often had but few and indistinct coils.

The spirochaetes were able to move in either direction and often reversed the direction of their movements; sometimes they showed active translatory movements and sometimes remained almost stationary whilst exhibiting the most vigorous lashing evolutions. At other times they lay stretched out almost in a straight line, their bodies deeply coiled, and exhibiting only helicoid movements. Jerky movements were seen frequently.

There appeared to be a periodicity in the movements. After showing the active movements for a time the spirochaetes quieted and then after a longer or shorter interval recommenced activity. During the interval they appeared to be motionless, were generally stretched out quite straight, and their bodies were always marked by a number of waves.

The pus in which the spirochaetes were examined contained a great number of polymorphonuclear leucocytes and epithelial scales. Living spirochaetes were never seen to enter the leucocytes although they were sometimes seen attached to them and apparently unable to escape, and in stained preparations a few were sometimes found in these cells together with granules which may have been the same organisms partly digested. Spirochaetes were, however, often observed in what appeared to be the act of boring into the epithelial scales. From the nature of the case it is impossible to be certain that the spirochaetes were actually entering the cell body, but when carefully focussed they were seen to be lying at the same level as the nucleus. In the cases watched continuously the spirochaetes advanced with one end towards the cell, appeared to penetrate it, made their way inside, and then became quiescent. The formation of coccoid bodies was not actually observed, but some of the cells contained strings of granules arranged in sinuous lines as if they had been formed within the bodies of spirochaetes. No spirochaete was seen to enter a cell and emerge again from it.

There were also bacteria in the urethral discharge and on one or two occasions contact between a spirochaete and a bacterium was observed to result in the most extraordinary twisting and contortion of the former. It was impossible to follow the movements owing to their lightning rapidity but for a moment the whole organism appeared to be involved in a violently agitated tangle until it was able to shake itself free. At other times spirochaetes collided with bacteria, cells, and one another without visible effect. These motions were similar to those referred to as "Catherine-wheel-like." Occasionally two spirochaetes were seen to become adherent to one another by their ends and to be unable to break apart. This event did not, however, suggest conjugation, but appeared to be more in the nature of an accident.

The spirochaetes lived a long time after removal from the body, and when kept in an incubator at 37° C. were sometimes still alive on the second day. One specimen collected at 9 a.m., still contained innumerable motile spirochaetes at 5 p.m., that is after eight hours, but the movements were not of the vigorous nature observed at first but were almost entirely rotatory. The undulant motion was slow and often inconsiderable and lashing movements had ceased. The spirochaetes were, however, still well coiled.

When killed by exposure, and especially after dilution of the pus

with saline solution, some of the spirochaetes seemed to lose their coiled appearance and looked like straight rods. The same change was observable in stained preparations but was less obvious owing to the fact that in many of the apparently uncoiled organisms it was really the depth of the spiral that was diminished and indications of coils were still visible (see fig. I, 67–70). But in such preparations and in smears made of materials in which all organisms had been killed by heat the great majority of the spirochaetes remained definitely coiled.

Skein-like twists of spirochaetes were found in the freshly drawn pus and also some large tangled masses composed of a great number of the parasites coiled together. On one or two occasions the membrane appeared to be visible in the living organism.

Transverse division was observed in the living state. A good many Y-shaped organisms were seen, the two divergent limbs being actively motile, sometimes both, sometimes only one, and the common stem, which was thicker, being rather more sluggish or quiescent. On watching these forms the limbs were seen to separate gradually as if they unwound themselves from the stem until finally there resulted two distinct spirochaetes united at the base by a delicate filament. The connecting filament then divided liberating the two organisms. These forms have been interpreted as a stage in longitudinal division.

Morphology. The spirochaetes were long sinuous organisms with a flexible body thrown into a larger or smaller number of waves. Although all somewhat similar in appearance they showed a considerable number of morphological variations. They stained well with all the ordinary reagents, and were Gram negative. With the Leishman modification of the Romanovsky technique they were coloured dull blue or violet. Gentian violet stained the organisms most intensely and was therefore used in the specimens from which the drawings to determine the length were made, but for details of morphology the Romanovsky methods were preferable. Gentian violet has the great disadvantage that it fades very rapidly at any rate in West Africa and restaining is seldom satisfactory. As an intense stain carbol fuchsine is about as good, but I am at present unable to say whether it lasts longer or not.

Length. Within certain limits the spirochaetes varied in length, the longest forms being recognisably pre-division forms composed of two organisms united by a fine filament. Three hundred spirochaetes taken as they came were drawn with the aid of a camera lucida at a magnification of 2000, and measured. Pre-division forms were

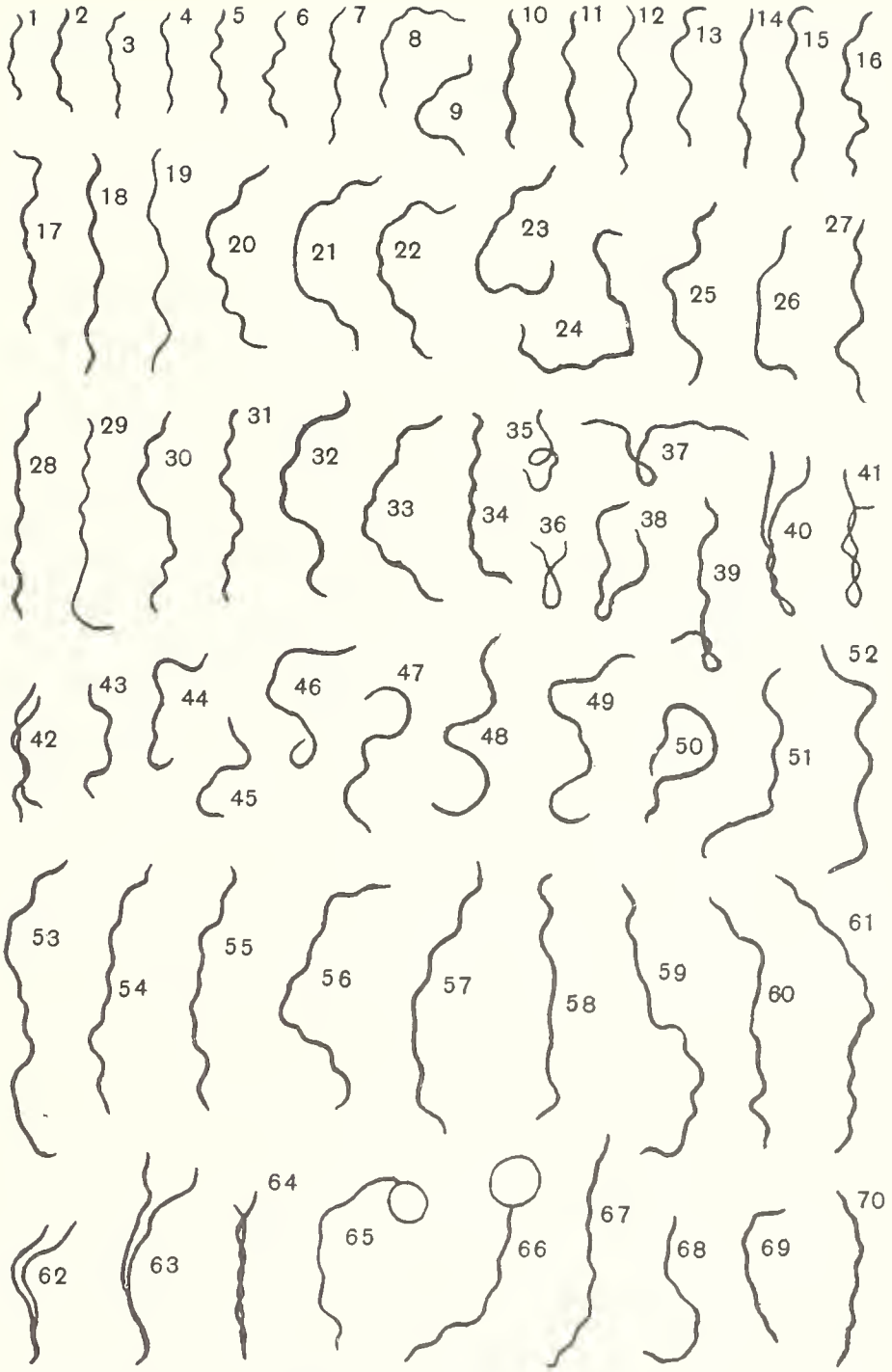

Fig. I. *Spirochaeta urethrae* Macfie. All the figures were drawn with an Abbé-Zeiss camera lucida at a magnification of 2000 diameters. (1–64), various forms found in the urethral discharge; (65–66), degenerated forms with cyst-like swellings; (67-70), spirochactes with indistinct waves found in specimens that had been allowed to die slowly at the laboratory temperature.

included. On the first day on which the patient was seen, that is on the second day after the discharge had started, a hundred spirochaetes were measured, and fifty on each succeeding day until the discharge ceased. The shortest of these three hundred parasites measured 5μ in length, the longest 20μ, and the average was 11μ (see Table I).

TABLE I. *Measurements of the length of* Spirochaeta urethrae *Macfie.*

Date	Number measured	Minimum length in microns	Maximum length in microns	Average
July 29	100	6	19	11·4
,, 30	50	7	19	11·18
,, 31	50	7	18	10·82
August 1	50	5	18	10·12
,, 2	50	7	20	11·50
	300	5	20	11·0

On distributing these measurements it was found that the commonest length of the spirochaete was 10μ, seventeen per cent. having been of this size, and that 65 per cent. of the parasites measured from 8μ to 12μ. It would be of very little assistance in recognising the spirochaete to know that it measured from 5μ to 20μ in length, especially as it is probable that both smaller and larger individuals would have been found if a greater number had been measured; but to describe it as an organism measuring most commonly 8μ to 12μ and sometimes being as short as 5μ or as long as 20μ would be a more serviceable definition. This statement is preferable also because it discounts the pre-division forms which really should not be taken into consideration since they are practically and often visibly double.

CHART I.

The distribution according to length of Spirochaeta urethrae *Macfie.*

In Chart I, these measurements of length are shown graphically. The curve is a striking one, with a well marked crest at 10μ, and shows most distinctly that the lengths of the majority of the spirochaetes fell within five microns of each other, between 8μ and 12μ. The curve is interesting also because it shows a slight subsidiary crest at 14μ. This is due to the inclusion of pre-division forms. These double forms, composed of two spirochaetes connected together by a fine filament, were frequently seen both in fresh and stained preparations. There was evidently considerable variation in the size attained

by the organism before it proceeded to divide, some of the double forms being made up of individuals 6μ or 7μ in length, and others of 9μ or 10μ. The two components of each double form were, however, always approximately the same size. Spirochaetes resulting from a recent transverse division might therefore measure anything from about 6μ to 10μ, and spirochaetes measuring 13μ or 14μ might already be in a pre-division stage. There would therefore be a length at about

14μ to 15μ which would include both well grown single forms and small pre-division forms, and I believe that it is to this over-lapping that the small subsidiary crest seen on the curve of lengths is due.

Breadth. Some of the spirochaetes were clearly broader than others, but so slender were the organisms that it was impossible to measure them accurately. So far as it could be estimated the breadth appeared to be about $0{\cdot}2\mu$ to $0{\cdot}3\mu$.

Waves. The bodies of most of the spirochaetes showed a number of curves or waves, but in all the preparations examined there were a few coiled forms and some simple curved or bow-shaped organisms without waves.

The actual number of waves varied. In the first place as was only natural the longer individuals possessed on the average more waves than the shorter ones, some of the pre-division forms showing certainly ten. A spirochaete of the average length, about 11 μ, had as a rule 4 or 5 waves.

The number of waves depended also to some extent on the thickness of the spirochaete as has been observed by Fantham. The relatively broad parasites generally had the body bent into fewer and wider curves than the more slender forms.

But even making all due allowance for length and thickness there still remained many variations. The rate of motion and the character of the movements at the moment of fixation probably accounted for most of these. As has already been pointed out the most actively moving spirochaetes showed vigorous lashing movements which obscured the curves of the body. Such parasites when suddenly fixed would presumably show a contorted outline, and in stained preparations it was in just such contorted spirochaetes that the waves were often fewest (see fig. I, 45–50). The less active spirochaetes, especially those showing only rotatory movements, were frequently stretched out in a straight line or slightly bent by undulant movements and showed a considerable number of waves. In stained films it was observed in accordance with this that the straight or but slightly bent spirochaetes showed the greatest number of waves.

In every preparation of the fresh urethral discharge there were a number of quiescent or dead spirochaetes, and by exposure to the temperature of the air or by heating all the organisms could be killed. These dead or quiescent spirochaetes always showed a considerable number of waves whether they were examined as wet preparations or after fixation and staining, but there was a marked difference between those that had been killed rapidly and those that had died slowly. Practically no difference could be made out between the spirochaetes killed by heat and those found in smears of pus obtained at the same time but immediately fixed and stained. In similar preparations which had been kept at the laboratory temperature, however, and in which all the spirochaetes had in consequence died (a process which took about eight hours), nearly all the organisms were linear in outline

and at first glance appeared to be without waves. A more careful examination showed that the waves were still there, and in the usual numbers, but that their amplitude was greatly reduced so that they were barely recognisable (see fig. I, 67–70).

Structure. The ends of the spirochaetes were almost always pointed, but some were rather blunter, and occasionally an organism was seen with one end more pointed than the other. The latter form is explained by Fantham as the product of a recent division.

The cytoplasm appeared to be homogeneous, and chromatin granules and rodlets occurred frequently at intervals along the body. A membrane or crest was seen in some of the spirochaetes quite clearly, but in the majority it could not be distinguished. One parasite with a double membrane was observed. The endoplasm in a few of the parasites was broken up into several pieces separated by narrow unstained intervals.

In not a few of the spirochaetes the body contained a number of deeply stained round or oval bodies that appeared to have been formed by concentration of the cytoplasm. These were presumably coccoid bodies. Some of the parasites appeared to consist of coccoid bodies only enclosed within a periplastic sheath.

Division. Whenever the spirochaetes were examined double forms (fig. I, 59–61) composed of two organisms united by a delicate filament were seen, and in a series of specimens it was possible to trace all stages between these forms and unattenuated individuals. In fresh preparations the separation of the central strand was observed to take place, two independent organisms resulting from this transverse fission. The process so far as it was observed resembled that described by Mackinnon (1909) for *S. recurrentis* rather than that described for *S. gallinarum* by Hindle (1911); although not infrequently parasites were seen doubled back and coiled round their own bodies (see fig. I, 39–41), and one or two intertwined couples were observed (fig. I, 42) such as Hindle mentions as resulting from the spirochaete breaking in two before the daughter parasites have uncoiled from each other.

On several occasions Y-shaped parasites were seen. The behaviour of these forms when alive has already been described. In stained preparations the united end did not show any indication of being composed of two spirochaetes twisted together and was quite distinct from the "incurved" forms, and I am therefore of the opinion that they represented a stage in longitudinal division. These forms were especially numerous in a 24 hour old culture made by adding some

of the urethral discharge to a tube of nutrient bouillon (fig. I, 62–64).

Division then appeared to take place both transversely and longitudinally, but owing to the rapidity with which the patient recovered the observations made were not so numerous as could have been wished and I do not feel justified in making any dogmatic statement.

Degeneration forms. In a tube of nutrient bouillon inoculated with the urethral discharge and kept in an incubator at 37° C. spirochaetes were still recognisable on the fifth day although no living ones had been seen since the second day. A number of these dead parasites showed the peculiar degeneration forms that have sometimes been described as "cysts"; that is to say large round cyst-like bodies had formed in them, generally at one end (see fig. I, 65 and 66). These cysts appeared to be empty, and were no doubt the result of plasmolysis (*cf.* Hindle, 1911).

The Intracellular Phase of the Spirochaete.

The discharge from the patient contained a great number of epithelial scales derived from the lining of the urethra. Some of these scales had a fairly healthy appearance, some seemed to be mere empty shells, and others were invaded by spirochaetes.

A large proportion of epithelial scales contained minute rounded or oval granules which I believe to have been coccoid bodies. Some of the scales contained only a few, others contained great masses of them. Similar granules were found free in the pus of the discharge. These bodies showed up as clear refractile spots in fresh preparations and after fixation stained well with the usual reagents but were decolourised by Gram's method. They were of about the same diameter as the coccoid bodies seen in the free spirochaetes and resembled small micrococci, but no such organism grew in any of the cultures made from the discharge.

Other epithelial scales contained spirochaetes. In fresh specimens these cells were a striking feature since many of them were literally packed with exceedingly active parasites. When these scales were carefully examined the spirochaetes were seen to be collected at the periphery whilst in the middle was the nucleus surrounded by a larger or smaller core of granular cytoplasm in which coccoid bodies were usually abundant (fig. II). The spirochaetes were very active and

appeared to be endeavouring to break through the cell membrane, and on one occasion they were observed to succeed in this attempt and to escape as active, independent organisms. It seemed probable therefore that the scales in the discharge which appeared to be empty shells were infected cells from which the spirochaetes had already escaped.

In living specimens it was impossible to study the spirochaetes in detail owing to their active movements and the manner in which they were crowded together within the cells, but in dried smears the scales became flattened out or were sometimes ruptured and had discharged the majority of the parasites, and many of these were suitable for minute examination. The majority of the spirochaetes were well

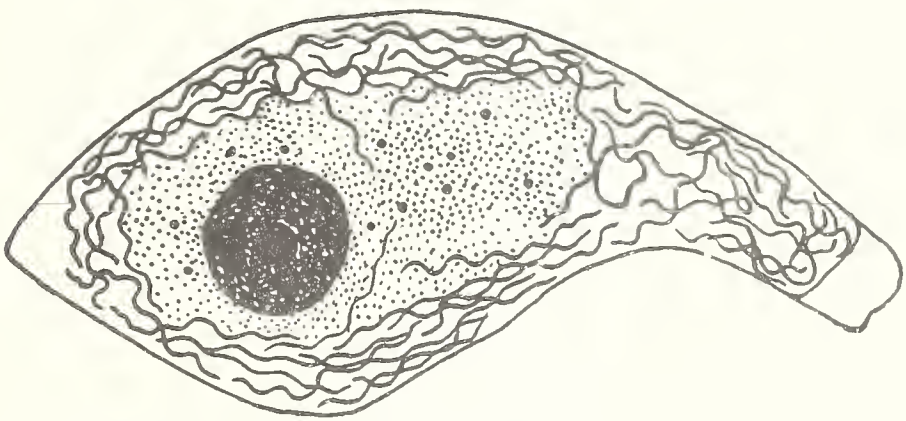

Fig. II. A urethral cell crowded with spirochaetes. Drawn from a living specimen with the aid of an Abbé-Zeiss camera lucida. Magnification × 2000.

developed and similar in size and morphology to the organisms found free in the urethral discharge. Others were very minute and all stages could be traced between a coccoid body elongated a little so as to be bacilliform to well developed spirochaetes with long slender bodies thrown into a number of pronounced curves. The very small organisms were relatively thick and had rather blunt ends. A few of these forms are shown in fig. III in one corner of a scale. The rest of the scale was crowded with spirochaetes and granules but they have not been detailed in the drawing.

The spirochaete therefore appeared to undergo a phase of development in the cells lining the urethra. In fresh specimens of the discharge spirochaetes were observed to penetrate into epithelial scales and to become quiescent in them. The actual development of coccoid bodies

from these spirochaetes was not observed but I think it may be presumed as other scales were seen containing chains of granules that had all the appearance of having been formed in spirochaetes. The next stage resulted in the formation of masses of coccoid bodies in the epithelial scales and this was probably brought about by the multiplication of the original granules developed in the spirochaetes since scales were seen showing every degree of infection from a single chain of coccoid bodies to great masses of the same organisms filling up practically the entire cell. The granules elongated forming first bacillary bodies,

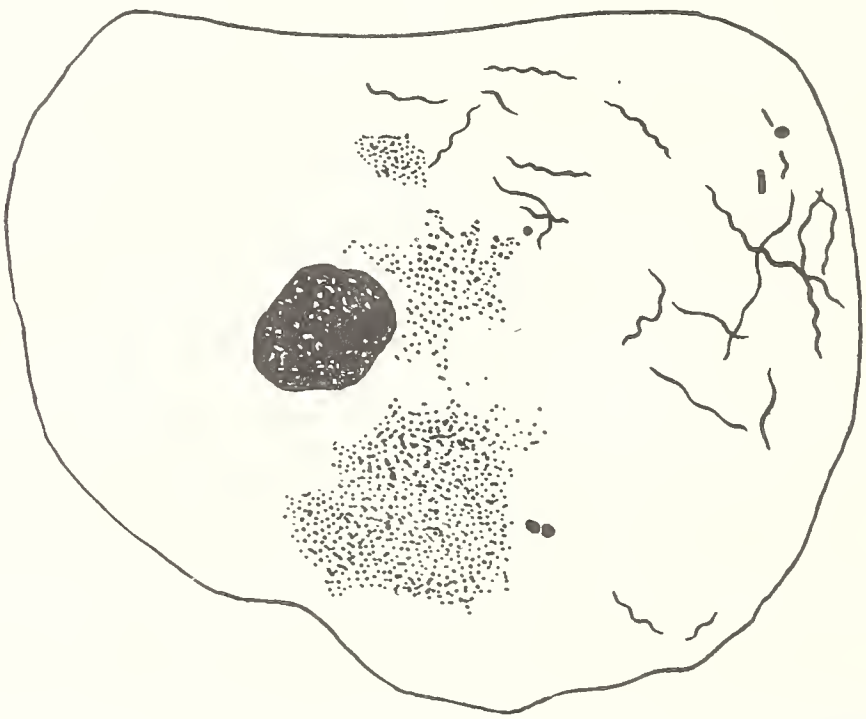

Fig. III. Spirochaetes in a urethral cell showing some of the stages of development from the coccoid granule. A few bacteria are also shown. Drawn from a stained preparation with the aid of an Abbé-Zeiss camera lucida. Magnification $\times 2000$.

then spirilla, then typical spirochaetes which grew and eventually escaped out of the cells and became free-living organisms. The phase of development would seem to resemble very closely that described by Hindle (1911) as occurring in ticks infected with *S. gallinarum*, but differed from it in this respect, that the spirochaetes remained within the cells until they had assumed the normal form instead of escaping in the state of short spirilla.

In the foregoing account I have endeavoured to indicate the actual facts observed before proceeding to outline the development which

the study of my whole series of examinations convinced me had taken place. I do not think a similar intracellular phase of any other human spirochaete has been described.

A very interesting controversy has sprung up with regard to the granule phase of spirochaetes which has been ably summarised by Fantham (1914). The formation of coccoid bodies has been observed in spirochaetes inhabiting the blood of vertebrates, the digestive tract and crystalline style of molluscs, the human mouth and intestine, etc., as well as in invertebrate hosts. Some observers have regarded these granules as degenerative, but their views have been criticised by Fantham (1914), and there can I think be little doubt that they are in reality developmental. They have repeatedly been described as growing into spirochaetes in the host, and *in vitro* the same development has been observed by Noguchi (1912) and Balfour (1913). In cultures by Bass's method of a spirochaete isolated from a guinea-pig's blood I have myself observed that subculture was successful when made at a time when no living organisms could be found but only granules, and degeneration forms.

Although in the vertebrate the part played by the granules in the case of spirochaetes possessing a natural alternation of hosts is not completely understood, in those species which do not show such an alternation they appear to be the cross-infective stage of the organism; and in the case of the spirochaete described in this paper the coccoid bodies found in the parasites in the urethral discharge were probably of this nature.

In the invertebrate host, the tick, Hindle (1911) described the spirochaetes as entering the cells before segmenting into coccoid bodies, whereas Balfour considered it was the granules which penetrated the cells. Fantham's (1911) account of the life cycle is apparently more in accordance with Balfour's view than Hindle's. The intracellular phase of the urethral spirochaete described above was I believe comparable to the development of *S. gallinarum* and other spirochaetes in the invertebrate host, and provided the organism with a means of rapid multiplication in addition to transverse and longitudinal division. The invasion of the urethral cells was probably effected in this case by the spirochaetes themselves and not by the granules, but once within the cytoplasm coccoid bodies were formed, multiplied, and developed into a fresh generation of spirochaetes. It was I think the occurrence of this intracellular phase which determined the onset of the urethritis.

The occurrence of groups of coccoid bodies liberated from spirochaetes has been observed in the cells lining the air passages in infections with *S. bronchialis*. Fantham (1914 and 1915) has interpreted these as being the cross-infective stage; but in view of the observations made on the urethral spirochaete described here it seems possible

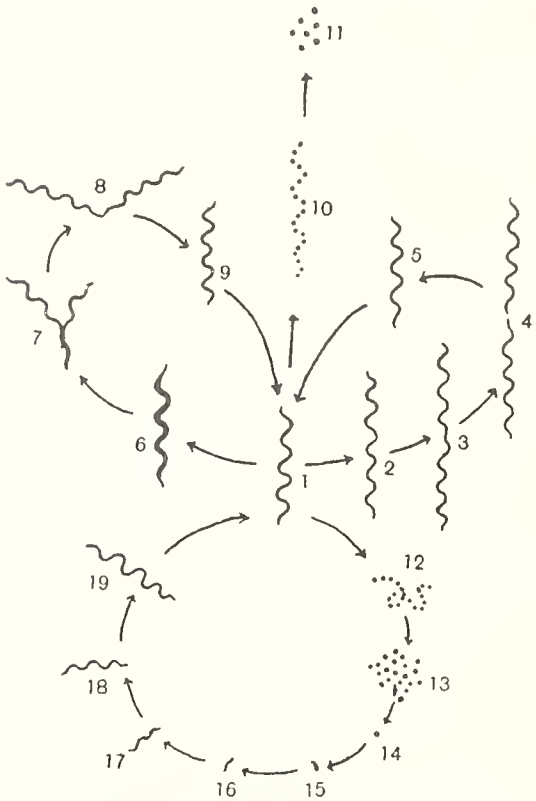

Fig. IV. Life-cycle of *Spirochaeta urethrae* Macfie (diagrammatic). (1–11), stages found in the urethral discharge; (1–5), transverse division; (6–9), longitudinal division; (10–11), cross-infection stage. (12–19), intracellular phase; (12), formation of coccoid bodies in a urethral cell; (13), multiplication of the coccoid bodies; (14), development of a coccoid body into (15) a bacillary form; (16), a spirilla form, and (17–18), small spirochaetes; (19), final stage of the intracellular phase, a spirochaete ready to escape from the cell.

that there may be a similar intracellular phase in the life-cycle of *S. bronchialis* and possibly of other pathogenic spirochaetes also.

Nomenclature. A large number of spirochaetes have been described in association with lesions of the genital organs, but so far as I am aware none has previously been observed in the urethra.

Urethral chancres are by no means rare in syphilitic infections and in such cases one would expect to find *Treponema pallidum* and

perhaps *Spirochaeta refringens* in the discharge. The spirochaetes found in this case had a membrane so that they were not treponematas, but this is not perhaps of such great significance as is supposed, and Fantham commenting on the observation of granules in *T. pallidum* by Balfour suggests that this organism may be "really a member of the genus *Spirochaeta*, too minute for observation of a membrane or internal chromatin granules, and so its coils may only *appear* to be fixed." In other respects also the parasites differed from *T. pallidum*, and as the clinical history, symptoms, and duration of the urethritis were not suggestive of a urethral chancre, I think the idea that the condition was due to syphilis may be dismissed.

Of the other spirochaetes associated with genital lesions *S. refringens* and *S. balanitidis* are those most closely resembling this organism. These two species are considered by some writers (Rille, Kraus) to be the same, others believe them to be distinct (Hoffmann and Prowazek), and Eitner, Richards and Hunt conclude that more than one species is included under the name *S. refringens*. There is some doubt whether either species is truly pathogenic, although Baermann has furnished evidence that *S. refringens* may spread beyond the superficial lesions, and Hoffmann and Prowazek believe *S. balanitidis* to be the causal organism of balanitis.

It is at least clear, I think, that the spirochaete I have described was not a mere saprophyte but actually invaded the cells lining the urethra setting up an acute inflammatory condition. The intracellular phase I have observed distinguishes the parasite from any of those previously described, and I therefore propose for it the name *Spirochaeta urethrae*.

A Note on the Measurement of the Length of Spirochaetes.

Spirochaetes undoubtedly vary very greatly and under different conditions assume different forms and show different types of motility so that it is difficult to select any characters or measurements that may be considered typical of the organisms.

According to Bosanquet (1911) Krienitz in 1906 found that in the case of *S. microgyrata* "the form of the organism changed with changing conditions, the alterations involving both length, thickness, and arrangement of curls" and that in consequence he "doubts the possibility of distinguishing spirochaetes by their morphological characters alone."

Fantham (1909) also has emphasized the morphological variations of spirochaetes.

Wolbach (1914) in recording some investigations on the cultivation of filterable spirochaetes, including one species found commonly in the human intestine, points out the cultural resemblance of these organisms to bacteria. In the case of those spirochaetes that can be cultivated therefore bacterial methods of differentiation might be of assistance. It is probably only in this way that mixed infections could be resolved and the risk of including in one description two or more dissimilar parasites entirely eliminated.

With regard to measurements of length the system of stating the range between the maximum and minimum observed in preparations obtained from various patients at different stages of the infection can commend itself to but few. Logically this method would include the minutest forms produced by the elongation of a coccoid body and the longest compound forms such as those measuring 140 μ found by Pollard in hospital gangrene. It would be more satisfactory to adopt as the length characteristic of the organism the length of the most common forms, this being equivalent to the bacteriological method of taking the average size of the organisms in a recent healthy culture. In the case of spirochaetes that can be cultivated this method would present no difficulties, and in others an approximation could be arrived at by measuring a large number of individuals and distributing them according to length much as is done in the case of trypanosomes. By measuring a large number of the spirochaetes in any particular case and plotting their lengths it would be possible to determine from an examination of the curve they formed whether it was a single organism or a mixed infection that was being dealt with.

The difficulty of deciding what is the characteristic length of a spirochaete was exemplified in the case of an organism I cultivated from the blood of a guinea-pig (1914)[1]. This parasite varied in length from 1 μ to 35 μ in different cultures at different stages, but in recently inoculated tubes (53 hours) in which the organism was growing actively they were much more uniform, the crest of the curve of measurements

[1] In view of the discovery of a somewhat similar spirochaete, *S. icterohaemorrhagiae*, in Weil's disease it is of some interest to recall that this guinea-pig was supposed to have been dying of yellow fever at the time the blood culture was made. The suggestions of Schaudinn and of Novy that yellow fever might be due to a spirochaete, the position of *S. interrogans* described by Stimson, and the possibility that Seidelin's *Paraplasma flavigenum* might be a stage in the development of a spirochaete, deserve further consideration.

occurred at 5μ, and nearly 70 per cent. of the parasites were from 4μ to 6μ long.

Another instance is *S. bronchialis* which has recently been studied by Fantham (1915) who has criticised some earlier measurements made by me. This spirochaete according to Fantham measures from 5μ to 27μ in length, but he admits that the "size of a number of them centres around 15μ, while many of the others are about 8μ long." The average length of my specimens was 8μ to 9μ and this Fantham attributes to the fact that they were obtained from two cases only in which he thinks "it was likely that the majority of the spirochaetes were at the same stage of development." I do not propose at present to discuss the morphology of *S. bronchialis* further as I hope to return to the subject later, but it is I think significant that my figure for the average length of this species is the same as one of the two commonest sizes given by Fantham and approximately half the other especially as "definite evidence of transverse division" was obtained in spirochaetes of the latter size. If then there is such an approximately constant form of the spirochaete this I submit should be accepted as the form characteristic of the species, as it would be the recognition of this type that would be of the greatest assistance in identification.

In the case of the spirochaete described in this paper satisfactory cultures were not obtained and I have therefore determined the length of the organism by measuring 300 individuals. The curve plotted from these measurements proves, I venture to think, the advantages of this method.

Summary.

(1) In the discharge from a case of acute urethritis spirochaetes were found which it is believed were the causal agent of the disease.

(2) The spirochaetes were most commonly 8μ to 12μ in length, and showed four or five spirals; but the range in the three hundred parasites measured was from 5μ to 20μ. They appeared to multiply both by longitudinal and transverse division and by the formation of coccoid bodies. The parasites passed through an intracellular phase which seemed to be as follows: some of the spirochaetes enter the epithelial scales lining the urethra, become quiescent, and break up into coccoid bodies. These bodies multiply so as to form masses of granules from which young spirochaetes develop, grow to about the normal size, and eventually escape.

(3) The name *Spirochaeta urethrae* is proposed for the parasite.

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STUDIES ON PEDICULUS.

I. THE COPULATORY APPARATUS AND THE PROCESS OF COPULATION IN *PEDICULUS HUMANUS*.

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(With Plates III and IV and 12 Text-figures.)

CONTENTS.

Introduction[1].

Owing to the active interest now being taken in all that concerns lice and their habits, especially in connection with the war, it seems expedient to forestall a fuller publication, now in preparation, by

[1] I am much indebted to Dr D. Keilin for his very able and friendly assistance in connection with the work upon which this paper is based.

giving an account of the copulatory apparatus and the curious process of copulation in *Pediculus humanus* Linn.[1]

It is remarkable, in view of the mass of literature that has been published on lice, dating from the time of Lecuwenhoek, that the subject of this paper has remained practically untouched. Where it has been approached, it has received scant treatment, and, as we shall see, the structure of the copulatory organs and mechanism of copulation have been misunderstood. This has doubtless been due to but a few casual observations having been made upon the living insects.

We may begin by considering the main differences shown by the sexes in *P. humanus,* for some of them have a direct bearing upon the subject in hand. These differences concern chiefly the general body form, pigmentation, the structure of the legs, what may be visible of the genitalia viewed externally, the distribution of the hairs upon the abdomen, and the abdominal musculature. A number of these points of difference have been dwelt upon by previous authors and they are summarized as follows:

CERTAIN ESSENTIAL DIFFERENCES IN THE ANATOMY OF THE SEXES.

(*Exclusive of the generative organs.*)

The Male. The *abdomen* of the male is rounded behind, the ventral surface curving dorsally and forward so that the sexual and rectal orifices come to lie dorsally (Text-fig. 3). The anus lies just in front of the sexual orifice; its position is best indicated when the insect defaecates whilst feeding or immediately afterwards. The anus is marked by a minute pit with a small ventrally situated chitinous plate overlapped by a dorsal integumentary fold. Immediately behind the anal plate lies the broad transverse slit forming the sexual aperture from which the

[1] To avoid confusion I append the following Synonymy:

Pediculus humanus Linn. 1758.

P. capitis de Geer 1778
P. cervicalis Latreille 1803 } lice from head.
P. consobrinus Piaget 1880

P. corporis de Geer 1778
P. vestimenti Nitzsch 1818 } lice from clothes and body.
P. tabescentium Alt 1824

Most authors recognize what they hold to be two species the head louse and clothes or body louse. These are, however, identical in all points of structure and can no longer be regarded as distinct, though they may show slight biological differences. The studies here recorded have been carried out on so-called "*P. vestimenti.*"

dark point of what we shall call the dilator[1] (Plate III) may, or may not, protrude in life or in preserved specimens. Basally is seen the basal plate (internal) upon which the dilator articulates, the same being visible by translucency through the body wall.

The postero-ventral abdominal wall, which is very convex transversely, is traversed by a number of accordion-like folds which allow of considerable extension of its surface (Text-figs. 1, 3). The male is usually smaller than the female, its abdomen is slenderer and more clearly segmented. Although both sexes show variable degrees of *pigmentation,* the male usually shows the deepest coloration. In well pigmented males the abdominal tergites appear as pairs of dark transverse bands, an appearance never seen in the female whose integumentary structure differs in this region. In the male, the *abdominal hairs* are fairly regularly disposed in rows corresponding to the body segments, whereas, in the female, most of the hairs are scattered about irregularly except on the last segments. A very marked sexual difference is observable in the structure of *the first pair of legs* in the male, these being also larger and more powerful than the others.

The longitudinal abdominal intersegmental muscles in the male (Text-fig. 3) are very powerful; dorsally, at the base of the abdomen, they fall into line with corresponding muscles arising in the thorax (M. levator abdominalis of Müller) and form a continuous chain of five pairs of muscles on each side extending backward to the last segment. The corresponding ventral muscles are confined to the basal part of the abdomen, extending from the thorax backward so as to include only the first four recognizable sternites.

The Female. We have already indicated some of the characters of the female in the preceding paragraphs. The abdomen is broader than that of the male and posteriorly it presents a bilobed appearance (Text-fig. 7). The anal aperture lies dorsally between the lobes beneath which is situated the vaginal aperture whose entrance is seen when the insect is viewed from behind or in ventral aspect. When thus viewed, the aperture is seen to be guarded ventrally by two incurved flattened processes, the gonopods, which are fringed with hairs and connected in the middle line by a fold of the integument surrounding a circular space. Anterior to the gonopods, which are more or less pigmented, a darkly chitinized and thickened area marks the region occupied beneath by

[1] We apply the term dilator to the structure described as the parameres by Mjöberg and by Cummings. All writers on *P. humanus* falsely call it the penis (*v. infra*, p. 296).

the vaginal pouch (Text-fig. 6). The first pair of legs, as in the immature stages, do not differ materially in structure from the others.

The arrangement of the longitudinal intersegmental abdominal muscles (Text-fig. 6) differs from that seen in the male. Dorsally there are five pairs of these muscles to each segment, but they are only present beneath the last three penultimate tergites. Ventrally there are corresponding muscles: three pairs (omitting two pairs of more obliquely directed lateral muscles) at the base of the abdomen in continuation of the ventral longitudinal muscles of the thorax, and there are five pairs of muscles situated posteriorly beneath the fifth and sixth segments.

To recapitulate, the great difference in the longitudinal abdominal musculature observable in the two sexes may be summarized as follows:

	Dorsal abdominal muscles are present under segments	Ventral abdominal muscles are present under segments
In the ♂	2, 3, 4, 5, 6, 7, 8	2 + 3, 4, 5, 6
In the ♀	6, 7, 8	2, 3, 5, 6

This arrangement of the musculature was first observed by Landois (1865) in the female and by Müller (1915) in the male; the enumeration of the segments is in accordance with that advanced by Müller.

The significance of some of the structural differences above enumerated will be considered presently.

HISTORICAL.

Regarding the copulatory organs of the male and female.

The male apparatus.

The first reference in the literature on lice to the male copulatory apparatus is that of Leeuwenhoek. In the English edition of his works (1807, p. 163) it is stated that he observed some lice in which he found what appeared to be a weapon of defence or hollow sting lying in a groove whence it was protruded when the insect was roughly handled. He conjectured that the "sting" was present in the male only but did not reach a final conclusion on this point. Gaulke (1863, p. 315; cited by Landois) took the organ for an ovipositor which served for laying eggs in the skin, whereas nearly all subsequent writers, including Landois (1865, p. 52), refer to this sting-like organ as the penis. Landois described it as wedge-shaped and flattened, as consisting of a long basal portion and a slightly bent distal portion. The margins are thickened and a slit-like gutter runs from the point back into the basal part. The organ is protruded through a transverse slit situated dorsally upon

the last abdominal segment and appears to be retractile into a tube lying within the body. This tube bears teeth, as does the vagina, the teeth pointing anteriorly. Landois' figure agrees with his description, both being partly right and partly wrong.

Patton and Cragg (1913, p. 540) somewhat vaguely describe the organ as "of a comparatively simple type" consisting of "two pairs of chitinous rods lying in front of each other in the terminal segment of the abdomen and articulated on a moveable joint." The posterior pair of rods converge behind, "are in close contact with one another, and convey the ejaculatory duct to the exterior between their inner surfaces." The musculature is complicated. This description is cryptic and mostly wrong. The authors' "anterior pair of rods" are what is termed the basal plate, the "posterior pair" are the parameres, or what in this paper we term the dilator. The authors apply the name of penis to both of these structures combined.

Pawlowsky (1907, p. 30) illustrates his paper by cross and longitudinal sections of the male which are more enlightening than his text He does not appear to have understood the mechanism any better than the preceding authors, to have made dissections, or to have studied the living insect. He refers to the teeth upon the "enveloppe de propulsion" which he thinks holds the "penis" in place in the vagina; what he terms the penis are the combined basal plate and dilator. Pawlowsky's figures are good, for he evidently made faithful drawings of what he saw.

Mjöberg (1910, p. 252), although he does not mention *Pediculus*, compared the male copulatory organs in Anoplura and Mallophaga. He is the first author who seems to have understood the structure in the allied forms which he studied.

Leaving the testes and vesiculae seminalis aside for future consideration, we find that Mjöberg states that these insects usually possess a copulatory apparatus consisting of (1) a long ductus ejaculatorius, (2) a basal plate (in some Amblycera represented by two long free rods), (3) parameres, either free or more or less fused (probably serving to widen the vulval aperture to allow the copulatory apparatus to enter), (4) a "preputial sack," studded with small chitinous knobs. In Anoplura and Ichnocera, the male carries the female on his back, introduces the parameres, then everts the preputial sack which bears an apical penis that penetrates into the female. The chitinous knobs upon the sack anchor the organ to the vaginal wall. Copulation appears to last a long time.

We are indebted to Mjöberg (1910, p. 226) for giving us a nomenclature of certain parts of the male copulatory apparatus in ANOPLURA and MALLOPHAGA. To repeat somewhat, these parts are (1) the *basal plate,* articulating distally as a rule with more or less free structures, the ductus ejaculatorius always running dorsally to the plate; (2) the *parameres* (a term taken from Verhoeff, 1903, pp. 113–170 (cited by Mjöberg), who used it in relation to Coleoptera), these being accessory parts articulating upon the distal portion of the basal plate; (3) the *preputial sack* which surrounds the penis and distal portion of the ductus ejaculatorius and appears usually to be attached to the distal part of the basal plate between it and the parameres. Mjöberg supposes that the preputial sack, like the penis, may have originated from the ninth and tenth intersternital cuticle. (In *Haematopinus suis,* Mjöberg noted the great length of the narrow ductus ejaculatorius and its uniform width, but failed to trace it into the penis.)

Müller (1915, pp. 41–43, figs. 24, 25 and plates II, III) describes and figures the male genitalia. Confining ourselves to the copulatory organs, we find that he describes the ejaculatory duct as entering a copulatory apparatus so complicated that "even Pawlowsky did not fathom it." Müller describes it as a deeply invaginated apparatus with chitinous rods, plates, and teeth, but gives no account of these structures. He refers to the parameres (what we call the dilator) as the penis and says that the term "preputial sack" is a misnomer; in his opinion it should be called the "sac interne," following Jeannel's nomenclature applied to beetles[1]. It is clear that Müller did not grasp the significance of the structure as a whole. He gives figures similar to those of Pawlowsky, a longitudinal section of the posterior extremity of the male abdomen, which is accurate but for the relation of the so-called penis to the sack. His interpretation of the function of certain parts is inaccurate. He denies that the sack contains the ductus ejaculatorius (as stated by Pawlowsky) and finds that the duct ends dorsally in the sack. We shall see presently that the duct runs dorsally to the sack when the latter is invaginated, but that it runs inside the sack when it is expelled.

Cummings (1916, p. 257), like Mjöberg, does not deal with *Pediculus.* He adopts Mjöberg's nomenclature of the parts of the male copulatory

[1] *Vide* Sharp, D. and Muir, F. (1912), "The comparative anatomy of the male genitalia in Coleoptera," *Trans. Ent. Soc. London,* p. 603. According to these authors, the sack is evaginated in Coleoptera: it may have spines pointing basally, these preventing its withdrawal while distended. It is clear that the apparatus in beetles, though very different and varied in construction, has points of resemblance, functionally speaking, with that in *Pediculus.*

apparatus and considers that the basal plate is probably derived from two longitudinal apodemes upon whose posterior ends articulate the two parameres. He prefers, however, to call Mjöberg's preputial sack "the mesosome." The large and extrusible end of the latter is said to be continuous with the ductus ejaculatorius, and distally it bears the penis, with frequently a splint on each side called the telomere, and beneath, the hypomere (terms applied, according to Cummings, by Waterston, 1914, *Ann. S. Afric. Museum,* x, 279, to Philopterid forms). At the proximal end are the endomeres, usually strongly chitinized bands or rods, one on each side, supporting the membrane of the sack of which they are only local thickenings. Cummings notes a suture in the middle line of the basal plate.

Judging from the instructive figures whereby Cummings illustrates these structures in different forms (*Linognathus, Trichodectes, Goniodes,* etc., but not in *Pediculus*) it appears to me that in *Pediculus,* Mjöberg's parameres are the parameres + endomeres of Cummings. Adopting for the moment the nomenclature of Cummings, I believe that in *Pediculus* we have a partial fusion of the endomeres distally and that they exceed the parameres in length, the latter being fused laterally to the endomeres and thus appearing merely as two small points (see Pl. III, figs. 1, 2). Cummings illustrates the extruded preputial sack in three species belonging to different genera, and it is clear that the function of this organ, though it varies in form in different species, is similar to that we shall describe in this paper for *Pediculus.*

Although not wishing to detract from the importance of the contributions of Mjöberg and of Cummings, neither of them afford evidence of these authors having studied the apparatus they describe other than in caustic potash preparations, for only the chitinous structures are referred to. The mechanism of the apparatus therefore remains to be described and an effort to do so is made in this paper.

The musculature of the male copulatory apparatus has received scant attention. Except for the protractor muscles which have their origin at the posterior and postero-ventral portion of the body wall and run forward to their insertions upon the margin of the basal plate, the musculature of these parts has not hitherto been understood. In addition to the protractor muscles just mentioned, Landois (1865, pp. 53–54) refers to retractors inserted dorsally upon the plate and arising from the inside of the last segment; he does not state whether they arise dorsally or ventrally upon the segment. Pawlowsky (1907, pls. IV, VI, figs. 19, 29–33) describes circular muscles about the ejaculatory

duct, and retractors running obliquely from the ventral body wall to the dorsal surface of the sack, these being the retractors of the sack (for which we use the term vesica) whose function we shall describe presently. Pawlowsky places the ejaculatory duct within the inverted sack, whereas it runs outside excepting where it traverses it at the base of the penis. Müller (1915), in his illustrations, refers to the "Bulbus Muskulatur des Penis," muscles called the retractors of the vesica in this paper; Müller figures them in a cross section as running dorsally to the basal plate. He states correctly (p. 43) that these muscles are inserted in the folds of the sack, but from the name he gives them it would appear that he did not apprehend their function.

The female apparatus.

A survey of the literature shows that the female apparatus has not hitherto been understood. Landois (1865, p. 50) refers to the presence of teeth upon the vaginal wall, these being seen by transparency through the surface of the body. He figures circular, longitudinal and oblique muscles surrounding the vagina (what we term the uterus), but the structure and shape of the vagina and its relation to the uterus escaped him. Pawlowsky (1907, pls. II, III, figs. 4–12) illustrates his paper by figures of cross and longitudinal sections of the female. He evidently made no dissections and his description is inadequate. His figures indicate, however, that the vagina is flattened dorso-ventrally, dentate internally; muscles arising from the ventral surface of the abdomen run backward to attach themselves upon the dorsal surface of the vaginal wall; the proximal half of the ventral wall of the vagina has inserted upon it retractor muscles running obliquely forward to the ventral surface of the body and it is also traversed by a few muscle fibres which he terms sphincter vaginae. These muscles are not to be confused with others surrounding what we term the uterus but which Pawlowsky terms the vagina (he also terms these muscles sphincter vaginae).

Müller (1915, pp. 37–41, figs. 21, 22) gives an accurate figure of the posterior portion of the female abdomen. He terms the gonopods "vaginal palps" and refers to their position as being at the point of transition between the last two abdominal segments. He gives a misleading schematic figure of the female genitalia for he surrounds the uterus by circular fibres only, making it appear bulbous, the vagina being represented as a straight, narrow tube, whereas it is broad, purse-like, and dentate internally. Finally may be mentioned a superficial paper by Peacock (1916, p. 39) who describes the external features of

the female. He asserts that the gonopods serve to hold the penis during copulation, but this appears to be merely an inaccurate assumption on his part, we have failed to discover their ability to grasp any part of the male genitalia.

THE AUTHOR'S DESCRIPTION OF THE COPULATORY APPARATUS.

The Male Apparatus.

To obtain a clear conception of the mechanism of the parts, it is best to commence by giving a general description of the apparatus as it appears when fully extruded by the male in the act of copulation.

When a pair in copula are separated forcibly by traction with two pairs of forceps which hold their bodies, the extruded apparatus of the male appears to the naked eye as a large, pale, rounded mass protruding dorsally from the end of the abdomen. If the male is not molested, this mass is presently retracted into its body.

To view the extruded apparatus at leisure, it is necessary to kill the male by a rapid method, either killing the male alone, immediately after its removal from the female, or together with the female with which it is in copula. For this purpose the insects are best killed by immersion in water heated to 70° C. whilst they are held with a pair of forceps.

General description of the extruded apparatus.

Assuming that a male has been killed immediately after its separation from the female in the manner described, the insect, or the isolated apparatus, may be conveniently studied whilst floating in water. Or, if permanent preparations are desired, the parts may be (1) treated in the usual way with caustic potash and subsequently mounted in balsam, or (2) immediately fixed in Carnoy's solution and stained *in toto*, or (3) sectioned.

Chitinous structures: We shall confine our attention in the first instance to the chitinous structures. In a freshly killed male, viewed from behind whilst in water (Text-fig. 1), the apparatus is seen to consist of a thin-walled globular sack (*V. pen.*) supported upon a stem (*V. pen. S.*) issuing from between two darkly chitinized rods (*Dil.*) which are pointed backward and downward; these structures articulate upon what appear to be two dark rods (*B. P.*) which protrude from the last abdominal segment. The greater part of the sack, which measures

0·4–0·7 mm. transversely, is studded over with short chitinous tuberosities or teeth that point outwardly and basally; some areas, as shown in this and succeeding figures, appear finely granular and devoid of teeth. Protruding from the left side of the vesica, and this position appears to be constant, there is a darkly chitinized tubular structure, the penis (*Pen.*). The latter is continuous basally with the walls of the vesica and with a peculiarly shaped rod (*St. pen.*) imbedded in the wall of the sack.

When viewed laterally, as shown in the semi-schematic Text-fig. 2, the relations of some of the chitinous structures enumerated will be more

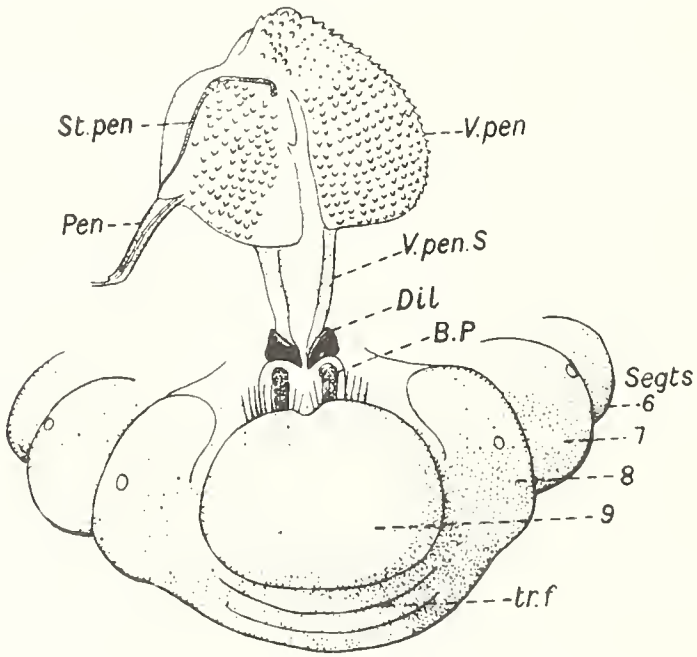


Fig. 1. *Pediculus humanus* ♂. Extruded copulatory apparatus seen from behind in a male forcibly removed from a female whilst in copula and promptly killed.

clearly understood; they are represented in solid black. The sack or *vesica* is represented by a serrated contour denoting the teeth which project from its surface, it issues from a cleft in the *dilator* (*Dil.*), the cleft being, however, better seen in subsequent figures. The teeth on the surface of the vesica are omitted for clearness' sake, the *statumen penis* (*St. pen.*) and *penis* (*Pen.*) are represented in black. The structure of the dilator will be best understood by reference to Plate III, figs. 1 and 2, wherein it is shown as it appears when retracted within the body of the male (fig. 1) and when extruded (fig. 2). The dilator is sharply

V-shaped distally, and, when extruded, it is flexed forward and downward so that its point impinges upon the dorsal surface of the male's abdomen.

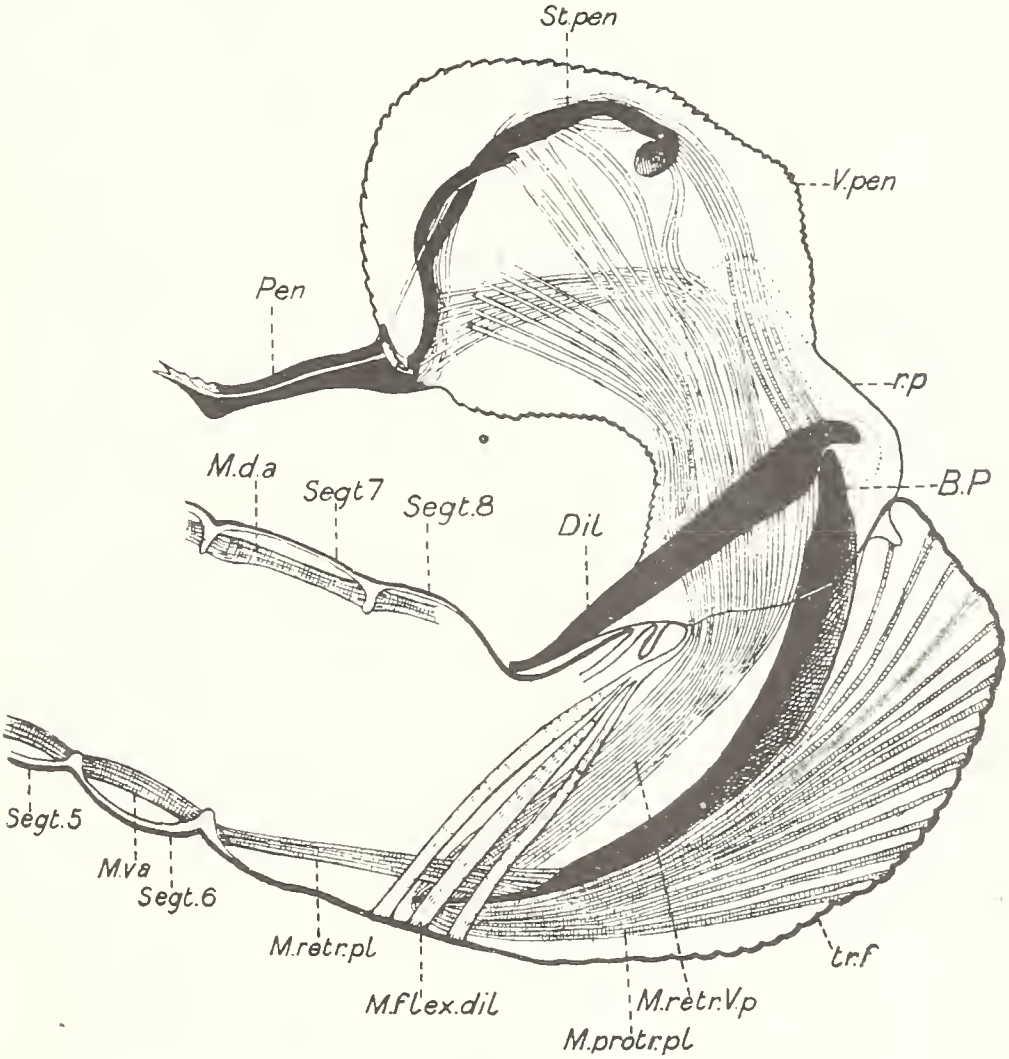


Fig. 2. *Pediculus humanus* ♂. Extruded copulatory apparatus seen from the side. Semi-schematic figure. The extruded parts, drawn from a carmine-stained preparation *in toto*, show the distribution of the muscles in the vesica penis (*V. pen.*): to the walls of the sack, the statumen penis (*St. pen.*) and penis (*Pen.*), the dilator being flexed and impinging upon the dorsal surface of the abdomen. The lower part of the abdomen is schematized from sections and dissections.

We may now consider in greater detail some of the chitinous structures which constitute the apparatus.

The component parts of the apparatus.

The copulatory apparatus of the male, when at rest, is usually completely retracted within the last three abdominal segments (Text-fig. 3). It issues through a large transverse slit upon the dorsal surface of the abdomen immediately behind the anus. The walls of this slit form the beginning of a flattened tube which is continuous with the chitinous exoskeleton. At its deepest point of invagination into the body, the tube folds backward and inward like the partly inverted finger of a mitten, and its walls fuse distally with the chitinous structures of the evertible copulatory organs (see Text-fig. 3 where the post-genital [*p. g. f.*] and pregenital fold, in front of *p. pl.*, are shown).

The essential parts of the apparatus are: (1) the *basal plate*, (2) the *dilator* (parameres), (3) the *vesica penis*, including its rib or strut, *statumen penis*, embedded in its wall, (4) the *penis*, and (5) the *ductus ejaculatorius*.

(1) *The basal plate* (Pl. III, figs. 1, 2; Text-figs. 1–4, *B. P.* or *B. Pl.*) is of elongated form, concave dorsally in transverse section, and curving gently in a dorsal direction along its length. It measures 0·75–1·15 mm. in length depending upon the size of the male (the other structures below-mentioned vary correspondingly in size). The basal plate is greatly strengthened laterally by a rod-like thickening on each side which arises gradually and terminates distally in a heavily chitinized rounded extremity. The pair of rounded ends articulate with the dilator which hinges upon them. Proximally the plate is bluntly rounded, thin and almost colourless, slightly rugose near the margin for the insertion of muscles; signs of a median suture are usually discernible. Distally, as is seen in sagittal sections (Text-fig. 3, *B. Pl. cl.*), the basal plate splits into two lamellae which are in continuity with the chitin forming the post-genital fold (Text-fig. 3, *p. g. f.*).

(2) *The dilator* (Pl. III, figs. 1, 2; Text-figs. 1, 2, 4, *Dil.*). Apart from lateral thickenings of the basal plate above described, the dilator constitutes the most heavily chitinized part of the whole apparatus. The structure is composite in origin for it is derived from two if not four chitinous rods (the *parameres* of Mjöberg) which through fusion of their distal extremities into a point have, functionally speaking, become a single V-shaped organ. The two rods diverge basally to their articulations on the basal plate. For purposes of description the name dilator is here preferred, it being more convenient to refer to the structure in the singular. The dilator measures 0·4–0·7 mm. in length. When the

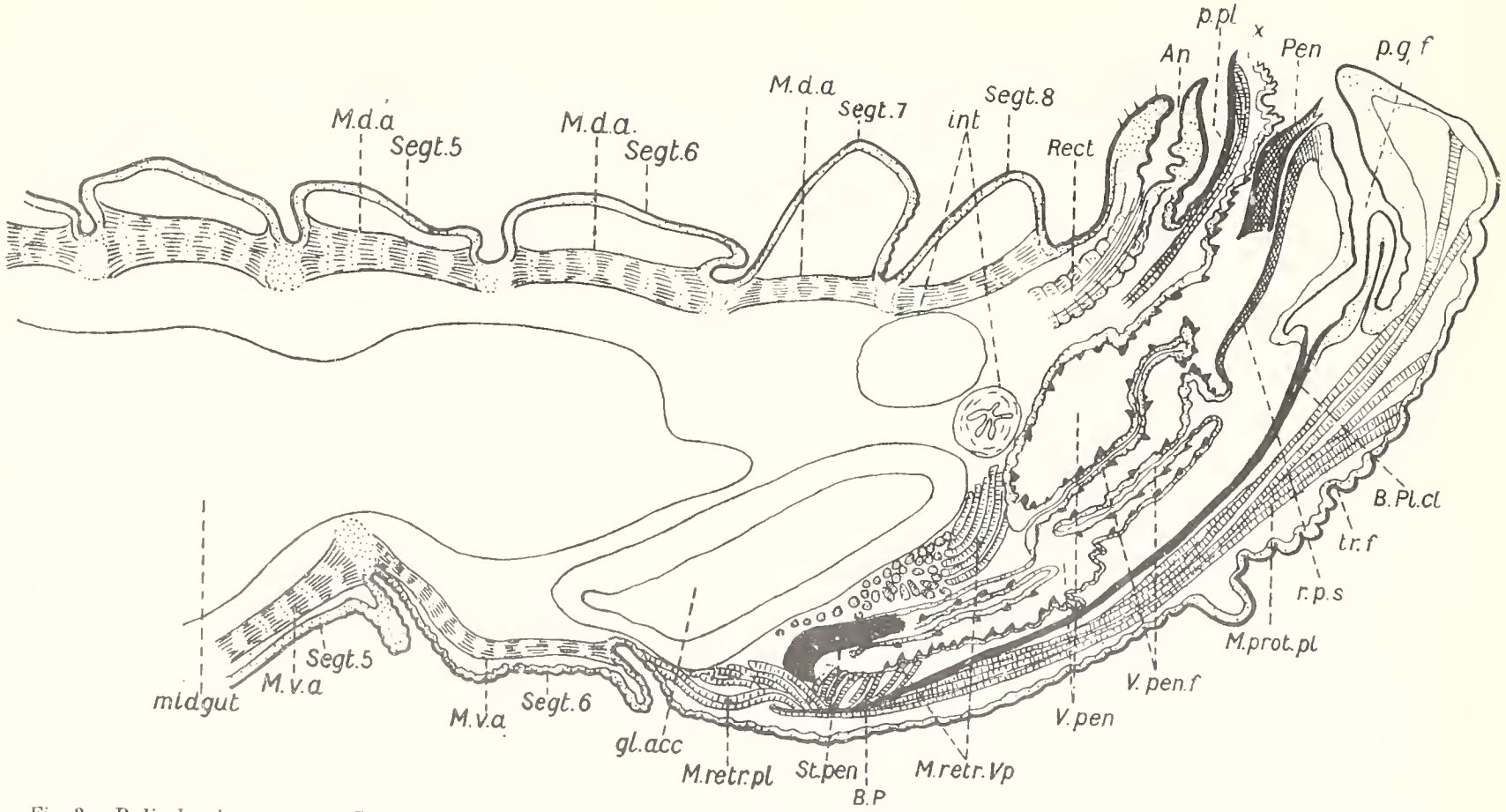


Fig. 3. *Pediculus humanus* ♂. Longitudinal, almost median section of the posterior part of the abdomen in a male whose copulatory apparatus is retracted into the body and at rest. The point of the penis is shown issuing from the inverted vesica, it has been somewhat displaced outwardly in sectioning. The thick chitin of the dilator has been torn away, causing a break in the section at ×. Figure slightly schematized.

copulatory apparatus is retracted within the male's body, the rods approach each other leaving but a narrow slit between them distally, the interspace broadens considerably behind; it is through this space that the penis issues (Pl. III, fig. 1, *Dil. cl.*). The dilator, when at rest, as shown in the figure just mentioned, points backward and slightly dorsally within the animal, lying on a plane with the basal plate. The point of the dilator is slightly curved ventrally (the reverse when seen in copula). The dilator rods are stoutest basally, they become flattened dorso-ventrally as they approach the tip; they show longitudinal ridges, and near their bases carry a lateral, backwardly directed spine. The supposedly composite origin of the dilator from the parameres + endomeres of Cummings has been already referred to (*vide* p. 299).

When, as we shall see presently, the process of copulation begins, the first part of the apparatus to appear is the dilator (Text-fig. 5); it is flexed upon the basal plate at about a right angle. At the next stage the vesica is slightly everted and the basal plate protrudes slightly. The pressure exerted by the vesica and penis in being forced through the cleft of the dilator greatly enlarges the cleft. The union at the distal end of the dilator, however, limits the increase in the space, and owing to the shafts becoming flatter and weaker as they progress toward the point they yield to the pressure and become twisted out of shape to a greater or less degree as depicted in Pl. III, fig. 2.

It should be noted that the vesica, described below, is in continuity with the upper inner margin of the cleft portion of the dilator, and to some extent perhaps with the basal plate. The boundary formed by the margin of the cleft about the issuing vesica may be likened to the edge of a pipe-bowl which is continuous in contour with a soap bubble blown from it. In addition, when the dilator is extruded and flexed, what was previously the dorsal having now become the ventral surface, a delicate *collar-like sheath* becomes visible, extending upward out of the genital orifice and fusing with the under surface of the dilator; this sheath forms a tube through which the vesica and penis glide outward from the body. The structure is best seen in Text-fig. 5; see also Text-fig. 2 and Pl. III, fig. 2.

(3) *The vesica penis*, when retracted into the body, carries with it the *statumen penis* and penis, all of which come to lie dorsally upon the hollow of the basal plate as in a spoon (Pl. III, fig. 1). The teeth which stud the outer surface of the extruded vesica now appear internal (Text-fig. 3), and the walls of the vesica are thrown into numerous folds

(*V. pen. f.*) which appear at times very confusing in sections; the teeth are mostly directed toward the genital orifice. When the vesica is extruded it is seen to glide outward upon the basal plate (Pl. III, fig. 2) and the teeth alter their direction as illustrated in the figure. The penis is seen lying within the invaginated vesica, its distal extremity pointing into the cleft in the dilator (Pl. III, fig. 1, *Dil. cl.*), through which it is propelled. The base of the penis is continuous with the *statumen penis*. The latter may be regarded as merely a rod-like thickening of the vesica wall, it shows some variation in appearance and size in different individuals, it measures 0·45–0·70 mm. in length but is difficult to measure because of its curvature. Basally, the statumen protrudes more or less from the inverted vesica, at this point it is broad and twisted and shows faintly visible chitinous strands which indicate where muscle fibres are inserted; its shaft is slender and bandlike, about midway it sends off a retrograde flattened branch which curves about it, and distally, near the base of the penis, it shows a large asymmetrical protrusion which is best seen when the parts are isolated (Pl. III, fig. 3). The appearance of the vesica when extruded has already been described.

(4) *The penis* is a tubular structure, darkly chitinized, and tapering to a point, somewhat like that of a quill pen. Its length varies, measuring from 0·24 to 0·32 mm. in the specimens examined. It is broadest basally, where upon its internal surface there are fine granulations and striations where muscles are inserted. Its shaft tapers to about a width of 20 μ, and distally it ends in a fine point or points of very thin colourless chitin. Running through the lumen of the penis and clearly discernible is the ductus ejaculatorius. The penis protrudes from the left side of the evaginated vesica; its chitin is continuous with that of the vesica and statumen.

(5) *The ductus ejaculatorius* is a very thin-walled, colourless and structureless tube of remarkably uniform width from near the tip of the penis to where it suddenly becomes muscular near the proximal end of the basal plate. Within the tube of the penis it measures 5–6 μ, posterior to this it may measure 7 μ, but possibly this slightly increased width may be due to flattening. In dissections of the inverted vesica, the duct can be distinctly traced from the base of the penis directly through the wall of the vesica, along whose dorsal surface it afterwards runs backward to the accessory glands. Basally, its muscular investiture sets in abruptly and rapidly grows in volume, the strong musculature doubtless being essential to force out the spermatic fluid through the long and minute duct. In one specimen the duct measured 1·1 mm. in

length from the base of the penis to where the duct became muscular. The whole course of the duct is best shown in Text-fig. 4, but its position is also shown in Plate III, figs. 1, 2, *D. ej.* It is evident from what has been said that when the vesica is everted the duct follows the penis,

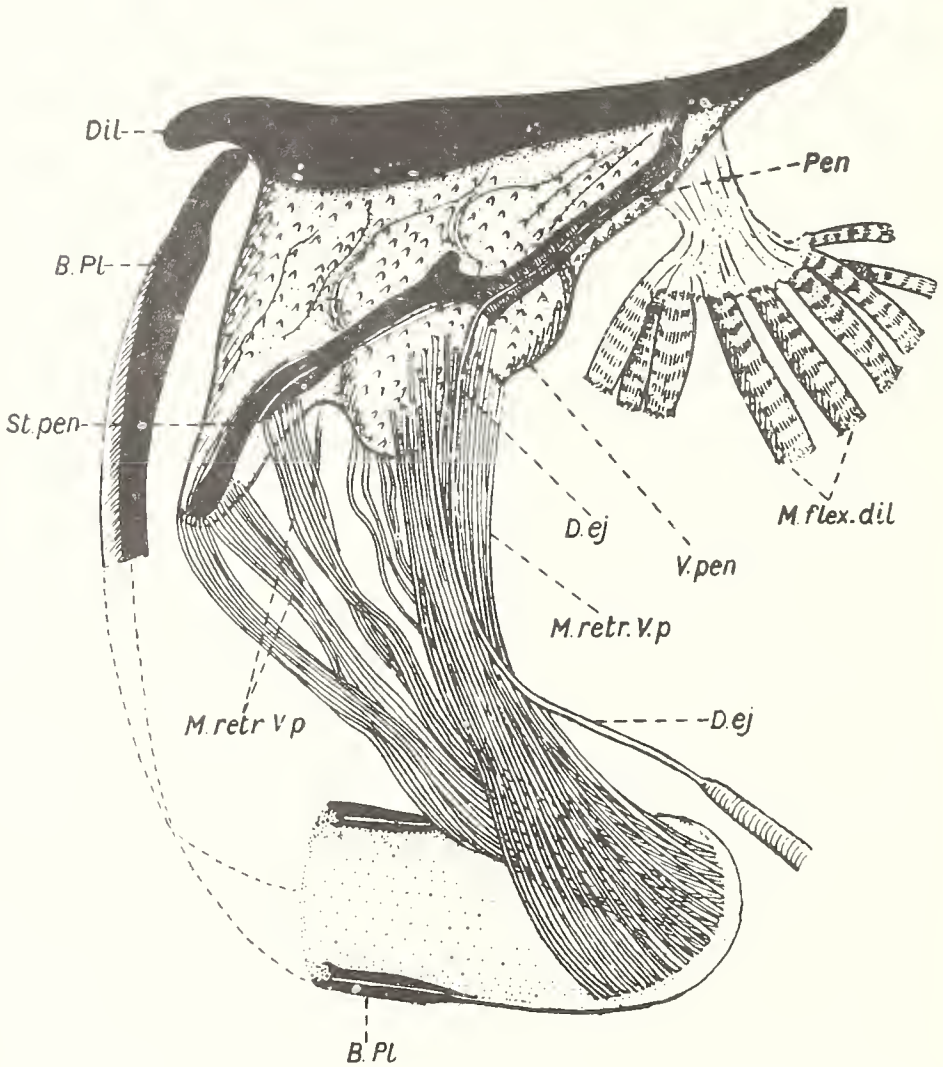


Fig. 4. *Pediculus humanus* ♂. Dissection of the retracted copulatory apparatus. The basal plate is cut across and the muscles running from its base to their insertions in the vesica, etc., are stretched. Figure slightly schematized.

traversing the vesica, where it runs parallel with the fibres of the retractor muscles which stretch outward in the cavity of the sack. The protrusive movement of the basal plate and whole copulatory apparatus, as is seen from sections, also entails a corresponding movement of the accessory glands which lie partly dorsal to the plate.

Mechanism.

In considering the chitinous structures we have already had to touch upon matters relating to the mechanism but there are other points still to consider, especially the mechanism whereby the apparatus is protruded and retracted.

The mechanism whereby protrusion is effected. When the living male is handled, as Leeuwenhoek and apparently no others since have observed, the point of the dilator is protruded to a moderate degree in the course of muscular efforts made by the insect to free itself. This point is the first part of the apparatus to be protruded, and males approaching females may be seen to protrude it intermittently in a similar manner. This movement is effected by a system of parallel muscular fibres, of great length, which arise at the posterior (ventral) part of the abdomen where it curves dorsally, and run forward to their insertions upon the ventral surface of the anterior or rounded end of the basal plate (Text-figs. 2, 3, *M. protr. pl.*). When a male is viewed ventrally, or the parts are dissected out, these protractor muscles are seen to cover the whole ventral surface of the plate, the fibres running parallel to each other, a few fibres running alongside the edge of the plate. When viewed in longitudinal sections the muscles are seen to spread out in a fan-like manner as they approach their points of origin on the wall of the abdomen. This wall, beneath the basal plate, is traversed by numerous creases which permit of considerable extension of the surface and only the deeper creases remain clearly in evidence when the copulatory apparatus is extruded (Text-figs. 1, 2, 3, *tr. f.*).

As the dilator is further extruded it becomes flexed upon the basal plate. This movement is no doubt in the main due to the traction exerted through the collar-like membrane connecting the dilator with underlying structures, mainly a system of powerful flexor muscles (Text-fig. 2, *M. flex. dil.*) which run obliquely backward and upward from the ventral abdominal wall to their insertion in the collar-membrane which serves as a tendon. These muscles do not, however, come into full play until a later stage, for the male starts the process of copulation by inserting the dilator only and it is not until the vesica is fully everted that he depresses the dilator fully by the action of these muscles so that the tip of the dilator rests upon his back. These flexor muscles of the dilator are only shown schematically, as in Text-fig. 2, as three fibres, whereas in Text-fig. 4, drawn from an actual dissection, they are shown torn away from their abdominal attachments and much contracted; the

collar-membrane in which they are inserted is partly torn away and lies external to the inverted vesica.

Following upon a moderate flexing of the dilator, whereby it forms roughly a right angle with the basal plate (Text-fig. 5; Pl. III, fig. 2) a third movement takes place. This movement concerns *the expulsion of the vesica,* and it is due to the flow of coelomic fluid out of the body cavity into the vesica, all of the retractor muscles being the while relaxed. The expulsion of the coelomic fluid which renders the sack turgid, is due to powerful contractions of the trunk muscles whereby the fluid is pressed out of the body into the vesica. It is possible that there are fine muscles present which serve to constrict the channel into the sack

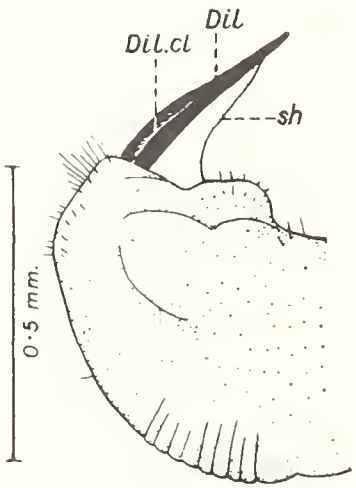


Fig. 5. *Pediculus humanus* ♂. Posterior part of the abdomen, showing the first stage in the protrusion of the copulatory apparatus, when the male attacks the female. Only the dilator protrudes and beneath it is seen the collar-like membrane which forms a sheath for the transit of the vesica, etc. Drawing from preserved specimen.

thus maintaining it erect, but if they exist they still remain to be discovered. It seems likely that there are no such muscles, and that the sack remains inflated once it has been filled with fluid, the turgidity being maintained throughout by the moderately sustained contraction of the muscles which reduce the space for the fluid in the body. When the vesica is fully extruded, the point of the dilator is fully depressed by the flexor muscles already referred to.

In the retracted apparatus (Pl. III, fig. 1), the point of the penis is directed outward toward the cleft in the dilator (*Dil. cl.*), the result being that when the vesica begins to issue (Pl. III, fig. 2, *Pen.*) the point of the penis promptly emerges from the cleft, its point being surrounded

by folds of the issuing vesica. The distortion in the form of the dilator due to pressure from the vesica has already been noted, that this pressure is very considerable is clear; we have one specimen in which a fracture of one of the rods of the dilator distally appears attributable to this cause.

Finally, the whole vesica disappears from the dorsal surface of the basal plate (Text-fig. 2); the neck of the sack is the last part to emerge (when inverted, it lies adjacent to the cleft in the dilator upon the ventral surface of the basal plate); the non-dentate or rugose portion (Text-figs. 2, 3, *r. p. s.*) is clearly seen in longitudinal sections.

The mechanism whereby retraction is effected. The *vesical musculature* is shown in Text-figs. 2, 3 and 4, and keeping in mind the description given of the process of expulsion of the vesica we obtain a fairly clear conception of how retraction is effected. To begin with, any pressure which may have been maintained upon the coelomic fluid in the vesica would have to be suspended, the flexor muscles of the dilator and the protractors of the basal plate would have to be successively relaxed. As is well shown in Text-fig. 2, the vesica is supplied with numerous very fine and long muscle fibres which arise around the anterior rounded margin of the basal plate upon its dorsal surface. These muscles run parallel to each other dorsally to the basal plate, and some of them, on entering the vesica, diverge in smaller bundles running respectively to the walls of the vesica, the statumen penis and penis base. The number of fibres is actually larger than figured, some fifty-six fibres having been counted in a cross section of the vesica. It is clear that when these muscles contract they will retract all the parts in which they are inserted, thereby causing the vesica to empty back its coelomic fluid into the body cavity while the vesica gradually becomes invaginated. It is possible that some fibres may give a certain independence of movement to the penis.

In dissections of the retracted copulatory apparatus, the retractor muscles of the vesica form a rounded mass, recalling a heap of string, lying dorsally upon the retracted vesica above the anterior end of the basal plate. In sections, the fibres run in different directions (Text-fig. 3, *M. retr. V. p.*), and from sections alone it would be impossible to explain their mode of action; these muscles constitute the so-called "Bulbus Muskulatur des Penis" of Müller (1915). The best picture of their arrangement is conveyed by Text-fig. 4 which represents a drawing from a dissection of the retracted copulatory apparatus. In this case the basal plate (*B. Pl.*) was cut across and the muscles running from its

base to their insertions were put upon the stretch between two fine needles, and, whilst stretched upon the slide, the preparation was fixed by dropping Carnoy's solution upon it; the specimen was afterwards stained and mounted in balsam.

The retractor muscles of the basal plate (Text-figs. 2, 3, *M. retr. pl.*) are relatively few. They arise from the ventral intersegmental fold bounding the seventh abdominal segment anteriorly and pass backward to their insertions laterally near the anterior end of the basal plate; these muscles will serve to retract the plate gently into the body.

The return of the dilator to its resting position is due to the relaxation of its flexor muscles and its elastic rebound upon its basal articulation; similarly, as the pressure of the vesica within the cleft lessens, the rebound of the chitin surrounding the cleft causes the lumen of the latter to narrow. When at rest, the axes of the dilator and basal plate fall into line.

The ductus ejaculatorius (Text-fig. 4, *D. ej.*) runs between the retractor muscles of the vesica and enters the latter at the base of the penis. The duct is therefore propelled passively with the sack when the latter is extruded, and it is also retracted passively.

Judging from the presence of a few muscle fibres attached to the posterior thickened wall of the pregenital fold (Text-fig. 3, *p. pl.*), these muscles may serve to occlude the lumen of the genital aperture and perhaps aid in the act of defaecation by raising and pushing forward the anal papilla.

The Female Apparatus.

The structure of the female copulatory apparatus is very much simpler than that of the male. Its position is shown in Text-figs. 6, 7 and 9. It is situated ventrally in the last three segments of the body. The vaginal orifice (Text-figs. 6 and 9, *vag.*) appears as a transverse slit occupying the greater portion of the width of the last segment, and immediately dorsal to it lies the anal orifice (*An.*) between the two terminal lobes of the body. In darkly pigmented specimens, a sharply defined, darkened area of thickened chitin of characteristic form, covers a part of this region ventrally (Text-fig. 6, *v. pl.*), and posterior thereto occur the paired, so-called gonopods which are usually pigmented (Text-figs. 7, 9, *Gon.*). The gonopods are in the form of flat incurved hooks which overlap slightly behind and whose inner concave margins are in continuity anteriorly through a concave fold of the integument; this fold and the inner margins of the hooks, bound a subcircular space

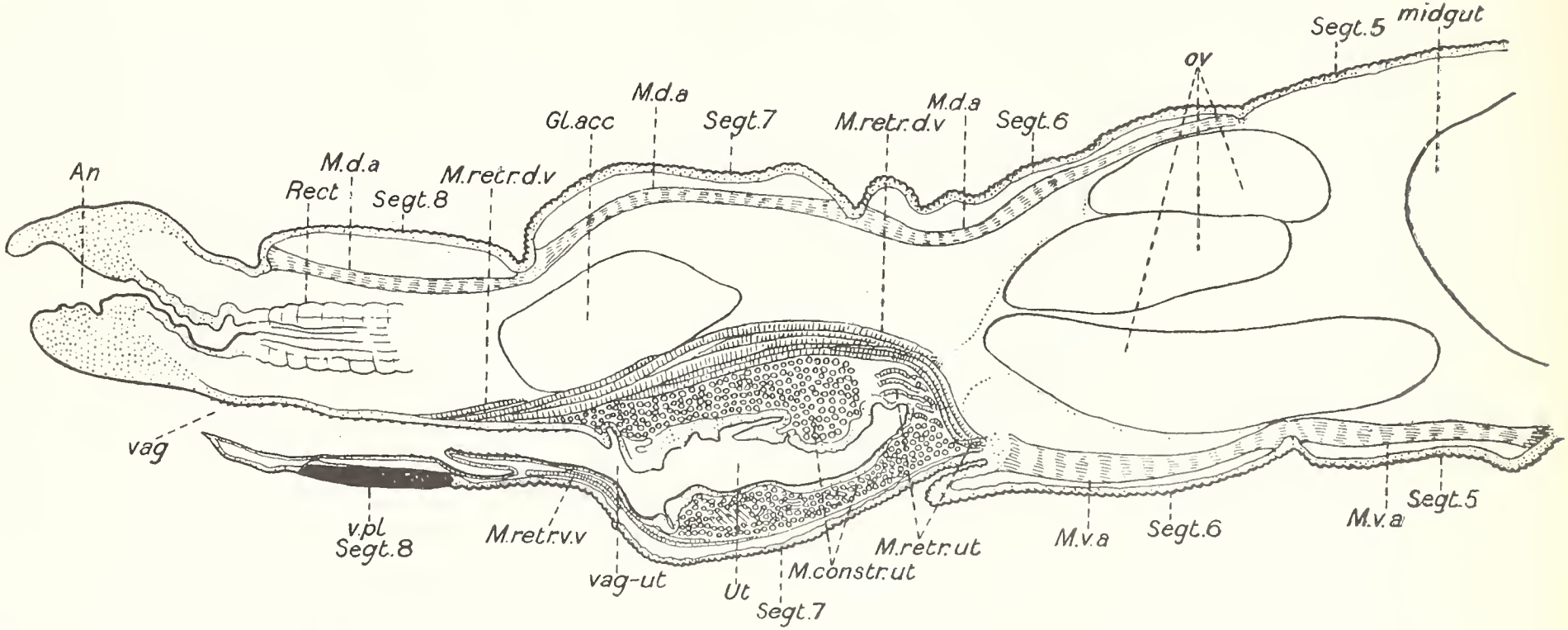


Fig. 6. *Pediculus humanus* ♀. Longitudinal, almost median section of the posterior part of the abdomen. Slightly schematized figure.

leading into the vagina. Projecting from the ventral margin of the vaginal orifice, and visible through the aforesaid space, is a chitinous flap (*v. fl.*) guarding the entrance. Folds of integument on either side of the orifice bear a number of fine long tactile hairs.

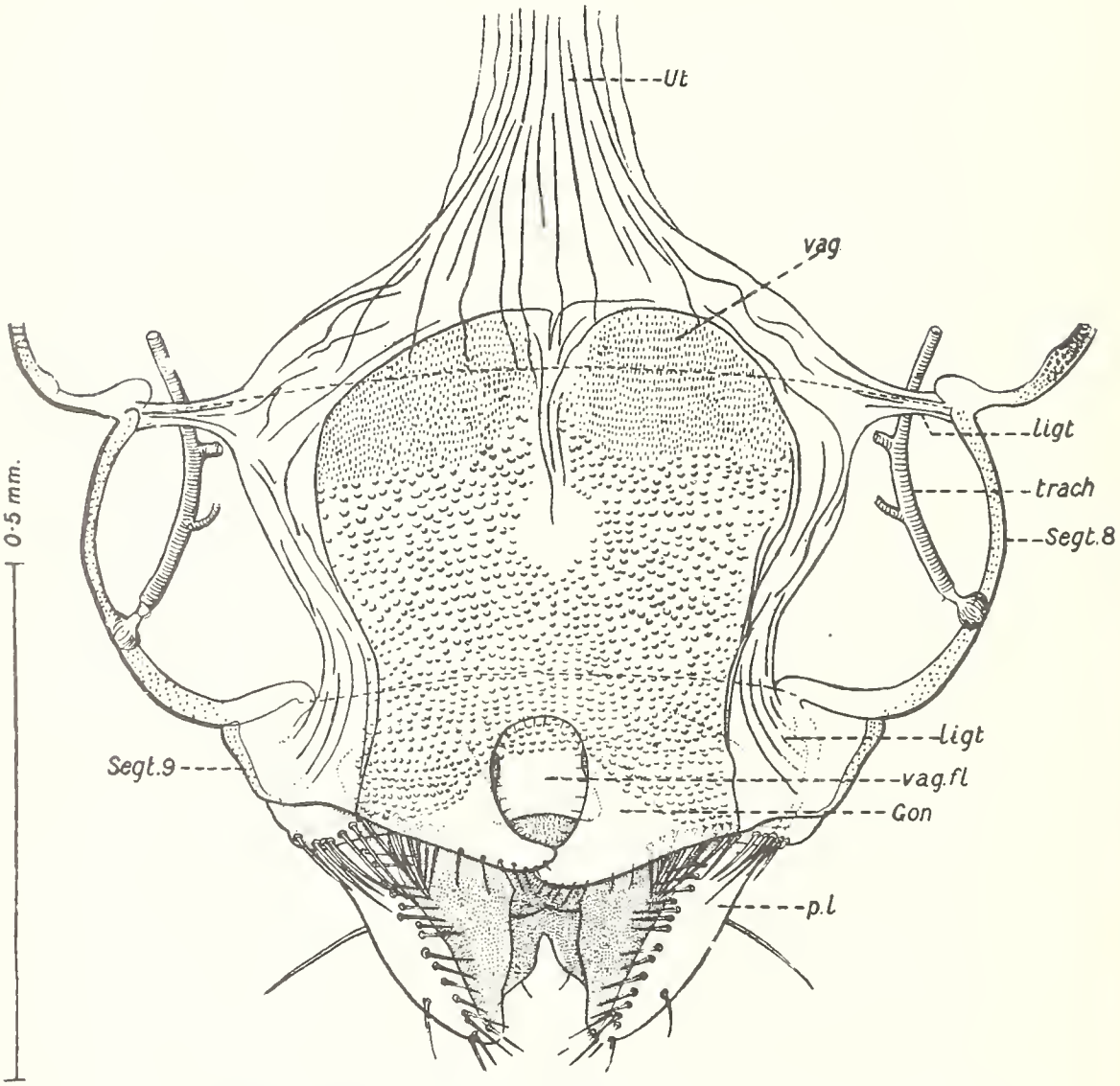


Fig. 7. *Pediculus humanus* ♀. Posterior segments of the abdomen in ventral aspect. Drawing from a specimen treated with caustic potash and mounted.

The vagina consists of a flattened purse-like sack, lying close to the ventral surface of the body; it measures about 0·5 mm. in depth and is covered on its dorsal and ventral inner surfaces by numerous minute

teeth which are directed outwardly (compare Text-figs. 6 and 7). The greater number of these teeth (Text-fig. 8) are semicircular in contour, these measure about 10 μ in width, but at the fundus of the vagina they are long and pointed, measuring about 10 μ in length; the distribution of the two kinds of teeth is sharply defined as shown in the accompanying illustrations (Text-figs. 7, 8). Laterally, chitinous ligaments (*ligts.*) running to the exoskeleton at the intersegmental folds, serve to hold the vagina in place. The vagina at its fundus leads into the uterus

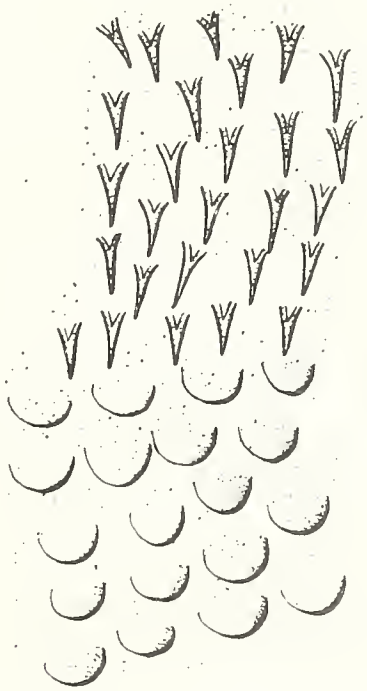


Fig. 8. *Pediculus humanus* ♀. Detail showing the dentition of the vaginal wall where the shape of the teeth changes abruptly as indicated likewise in Fig. 6. The pointed teeth measure 10μ in length.

(Text-fig. 7, *Ut.*) whose delicate and structureless chitinous lining is thrown into numerous deep convoluted folds allowing of great expansion for the passage of the eggs. The uterus is provided with a powerful musculature consisting, as Landois first showed, of transverse and oblique muscles surrounding its lumen (Text-fig. 6, *M. constr. ut.*); a consideration of the uterus does not, however, enter into the province of this paper.

The structures above enumerated can be seen to a great extent in living specimens, the teeth covering a part of the vaginal wall being visible also by transparency. Text-fig. 7 was drawn from a specimen

treated with caustic potash and mounted in balsam, it therefore only shows the chitinous structures.

Text-fig. 6 illustrates certain muscles that may or may not play a part in copulation: retractor muscles running from the posterior dorsal and ventral surfaces of the vagina (*M. retr. d. v.* and *M. retr. v. v.*) and running forward to their origin in the intersegmental fold bounding the seventh segment anteriorly. The retractor muscles of the uterus (*M. retr. ut.*) may also be noted, these also originate in the above-mentioned intersegmental fold and are more numerous than shown in the section, for they are mostly situated laterally to the median line. All of these muscles doubtless play an important part, together with the constrictors of the uterus, in the expulsion of the eggs. The function of the fine, backwardly directed chitinous teeth protruding from the

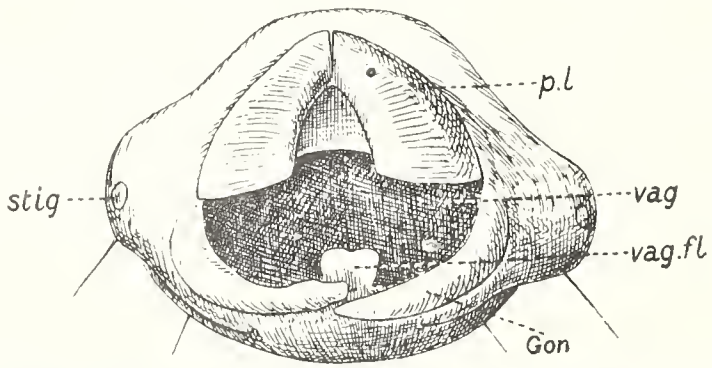


Fig. 9. *Pediculus humanus* ♀. Posterior aspect of a female killed whilst in copula and fixed, the male having subsequently been pulled away. Sketch.

vaginal wall is probably in the main to aid in the expulsion of the eggs, it is difficult to see how they could assist materially in copulation.

In Text-fig. 9 a sketch is given of the posterior aspect of a female which was killed and fixed whilst in copula, the male being subsequently pulled away. The vagina is seen to have been fully dilated by the male copulatory apparatus; the parts are lettered in accordance with the figures previously described.

THE ACT OF COPULATION.

Historical.

The first author to give any information about the process of copulation in *P. humanus* is Warburton (1910, pp. 23–27), who states that he observed a female copulating on the fifth day after ecdysis, after which copulation took place frequently and lasted for hours; he considered

that adults only begin to perform their sexual functions on the fourth to fifth day after ecdysis. He made rough sketches of pairs in copula but never published them. Sikora (VIII, 1915, pp. 523–537) observed that copulation may occur ten hours after ecdysis in lice kept at 35° C. upon man, and personally I have no doubt but that it may take place even sooner. Hase (1915, p. 64, cited by Müller) states that copulation lasts 40–70 minutes or more. Müller (1915, p. 43, and pl. III, fig. 8) is the first author who has illustrated a pair in copula: he gives their relative positions correctly in a coloured figure of a pair killed in copula with chloroform. Whilst he states that the fully extruded "penis sack" occupies the vagina, the teeth being directed outward and backward, his figure is incorrect as it only depicts the dilator entering the vagina. Finally, Hindle, in his unpublished notes made some two years before the outbreak of the present war, records that he observed copulation to occur whilst the insects were feeding, and that couples at times remained in copula for several hours.

The foregoing paragraph sums up all that has hitherto been published regarding the process of copulation. The paper by Müller became known to me through the courtesy of Dr A. E. Shipley, this autumn, after this investigation had been practically completed. Müller's description is, however, misleading, and, as far as I am aware, nobody has hitherto followed the whole process from start to finish or explained the anatomy and functions of the copulatory organs with even an approximate degree of accuracy.

The Author's Observations.

As the result of numerous observations made in the course of investigations on *P. humanus*, it has been found that the sexes may copulate at any time but that they do so mostly a few hours after feeding. To observe copulation, the insects require to be kept warm, otherwise they are inactive; a temperature of about 30° C. is suitable for the purpose. The following account is based on notes made immediately after a pair had been observed to enter into copulation, and it may be taken as a typical description of the sexual act. The observation was made with a Zeiss binocular microscope, magnification ×40, the tube of the instrument being placed horizontally so that the pair were viewed sideways and in a good light.

The male approached the female from behind whilst she was feeding, her abdomen being well raised behind. The male, with the claws of his

first pair of legs, seized the female's third pair of legs at the bases of the femurs. He then curved his back so that it became concave and closely applied to the body of the female. The female was thereby tilted into a vertical position with her abdomen curving away from the male. This led to the extraordinary position of attack depicted in Text-fig. 10, wherein the female stands as on a tripod consisting of her head and the claws of her forefeet which latter cling to the substratum and prevent

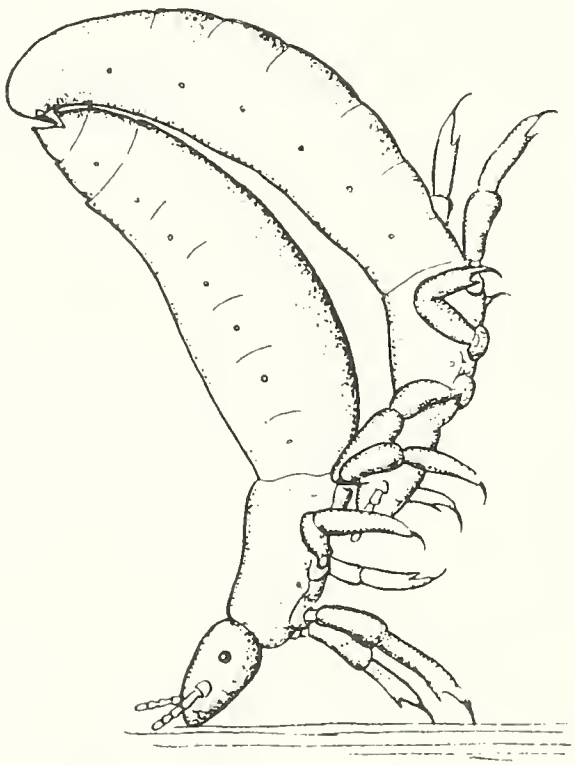


Fig. 10. *Pediculus humanus.* Commencement of copulation, the female stands tripodwise upon her buccal region and first pair of legs, entirely supporting the weight of the male who grasps her third leg-pair at the base with the claws of his first pair of legs, bends backward and inserts the point of his dilator into the vaginal orifice.

her performing a somersault. The male's whole weight is now supported by the female. The male maintains himself in position solely by his grip on the female's legs and by the slight support afforded from leaning backward against the slanting surface of the female's abdomen. During this time the male's head and antennae appear as if stroking the ventral surface of the female's thorax. The male, having extruded the point of the dilator and flexed that structure upon the basal plate (as in Text-fig. 5), protrudes and retracts it rapidly, but fitfully whilst seeking to

hook it into the vagina. Both sexes defaecate whilst these efforts continue, the amount voided by the female being greatly in excess of that voided by the male. The faeces, or rapidly inspissating masses of the freshly ingested blood are pushed to one side by the dilator as they get in the way, and finally, after a struggle lasting about a couple of minutes, the dilator penetrates the vagina. The dilator now gives the male an additional hold on the female and it is extruded still further and rapidly pushed in and out, whilst penetrating more and more deeply. After some 30–40 strokes, when it has penetrated to its full length, corresponding to the depth of the vagina, the parts are seen to glisten

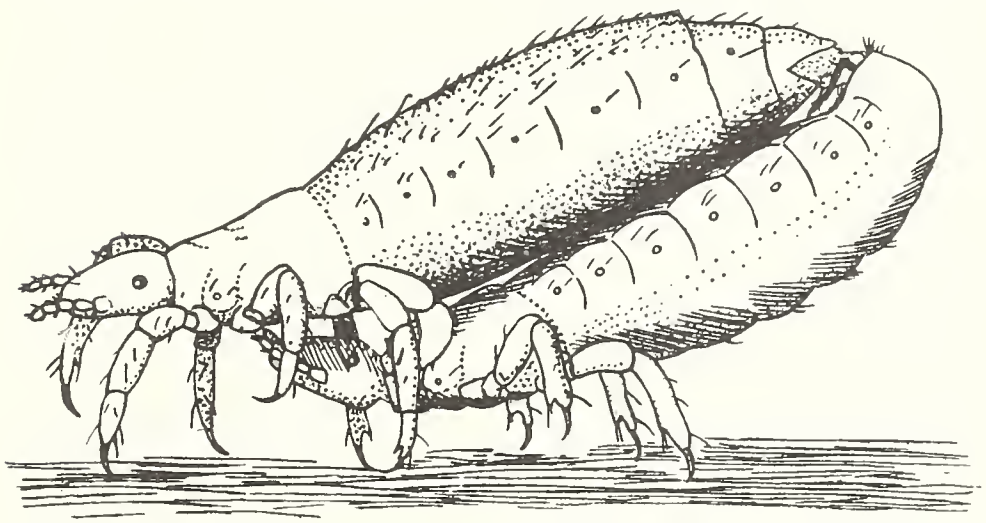


Fig. 11. *Pediculus humanus.* The male having dilated the vagina as shown in Fig. 10, has extruded his vesica with its distal penis into the vagina, and withdrawn the dilator whose point is flexed on his back. He is now firmly anchored by the teeth on the vesica adhering to the walls of the vagina, and the couple walk away to a retreat. This, and the preceding figure were drawn from hasty sketches subsequently amplified from killed specimens.

with secretion. The female's abdomen now suddenly shortens, owing to the last two segments becoming somewhat telescoped, apparently through the forceful efforts of the male. The dilator continues to penetrate deeply for a few strokes, it is then quickly retracted, having been rapidly replaced by the everted vesica. The dilator is immediately flexed downward and out of the way on the male's back, having served its purpose in widening the vagina. At no time do the so-called gonopods appear to come into play as graspers or otherwise. The dilated vesica fills the whole lumen of the vagina which it distends almost to

its own size, a small portion of the basal portion of the sack and the whole of its stem remaining outside the greatly stretched vulva. As soon as the vesica is safely anchored by means of its teeth to the inside of the vagina, the male ceases to strain backward, the female's abdomen again lengthens and the pair drop into a less acrobatic attitude upon their legs as shown in Text-fig. 11, and, united in copula, they retreat beneath a sheltering piece of cloth.

This undoubtedly represents the usual procedure. On one occasion only was a male observed to seize the female by the bases of the second pair of legs. When on cloth and ready to copulate, the females are frequently seen to assume an attitude approximating to that shown in Text-fig. 10, the hind pair of legs and the abdomen being raised almost vertically as if inviting the male to approach and seize her. The pair remain united for a variable time, anywhere from half an hour to several hours. A male which has just ceased to copulate with a female has been seen to clamber on her back, seize her about the body with his six legs, she walking away with him. At other times the male has been seen to copulate again immediately after having abandoned a female.

We may revert here to the differences already noted in the abdominal musculature of the sexes, since in part it bears directly upon the process of copulation. The more powerful dorsal system of longitudinal muscles in the male (compare Text-figs. 3 and 6) appears to be correlated with his ability to overcome the female in the effort to introduce his copulatory organs by arching his abdomen backwards and forcing the dilator forward; doubtless these muscles also serve to keep his abdomen from being unduly pressed downward by the female's weight during copula, especially when the pair move about. The latter suggestion as to the function of these muscles emanates from Müller who was the first to appreciate the differences in the musculature in the sexes.

The legs as accessory organs of copulation. Previous authors have noted the form and greater size and especially the powerful claws of the first pair of legs in the male. It is clear that they are more powerful, so as to enable the male, as we have seen, to grasp the female firmly during copulation. There is, however, a corresponding adaptation in the structure of the third pair of legs in the female. But for two drawings of the female by Terzi (in Castellani and Chalmers, 1913, pp. 632–633, figs. 268, 270) the structure appears to have escaped the notice of previous observers; its significance has certainly not been understood hitherto. Although less pronounced in poorly chitinized females, the structure is invariably present. It consists (Text-fig. 12) of a blunt

recurved ventral spur near the base of the femur. This spur serves to facilitate and maintain the grip of the male claw upon the leg.

In conclusion, a glance at Plate IV will serve to recapitulate the main points regarding the structure and interrelations of the copulatory apparatus in both sexes of *Pediculus humanus*. The figure is printed in two colours to differentiate the parts belonging to each sex, black for the male and red for the female. The figure represents a camera lucida drawing of a specimen mounted in balsam and confines itself to the chitinous structures. The terminology of the parts will be found accompanying the other figures described in the text.

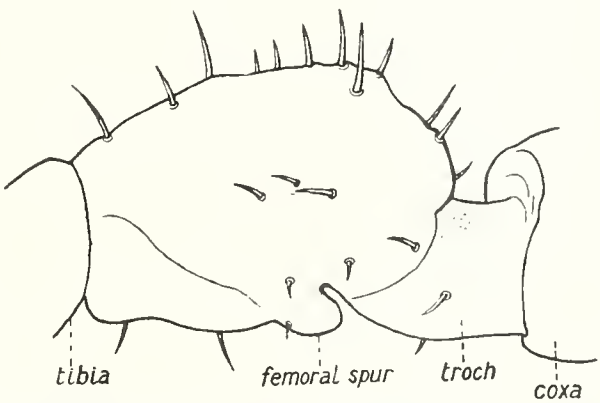


Fig. 12. *Pediculus humanus* ♀. Portion of the third leg, showing the ventral spur upon the femur which aids the male to grasp the female. Camera lucida drawing of the leg in profile.

The manipulations of killing and mounting the insects led to the male relaxing his hold with his claws, and, in consequence, their bodies became somewhat separated, the dorsal surface of the male and ventral surface of the female being uppermost in the illustration. The copulatory apparatus of the male occupies the greater part of the lumen of the vagina, although the male organ has been somewhat retracted. The parts of the male lying within the vagina are stippled and the teeth covering a corresponding area on the surface of the vaginal wall are omitted to avoid confusion. It may be regarded as certain that the vesica fills the whole vagina in life and the presumption is justified that the penis enters the lower part of the uterus. The illustration further elucidates some details in the structure of the male apparatus. Dotted lines indicate the course of the ductus ejaculatorius from above the dorsal plate through the vesica to the tip of the penis, dorsal to the

duct is shown the course of the anus to the rectum which opens immediately in front of the genital aperture.

Note on the Nomenclature of Parts.

In the foregoing description I have used some of the terms established by previous authors; most of these require no comment. The names applied to certain muscles are in accordance with their obvious functions as described in this paper. Several structures have been named for convenience of description and a few of these call for remark; namely the terms dilator, vesica penis and statumen penis:

Dilator: this term is frequently used to denote the *parameres*, which, through the fusion of their distal extremities into a point constitute, functionally speaking, a single organ. The dilator corresponds to what all writers except Mjöberg and Cummings call the penis or a part of the penis. It is a composite structure whose derivation is discussed on p. 299.

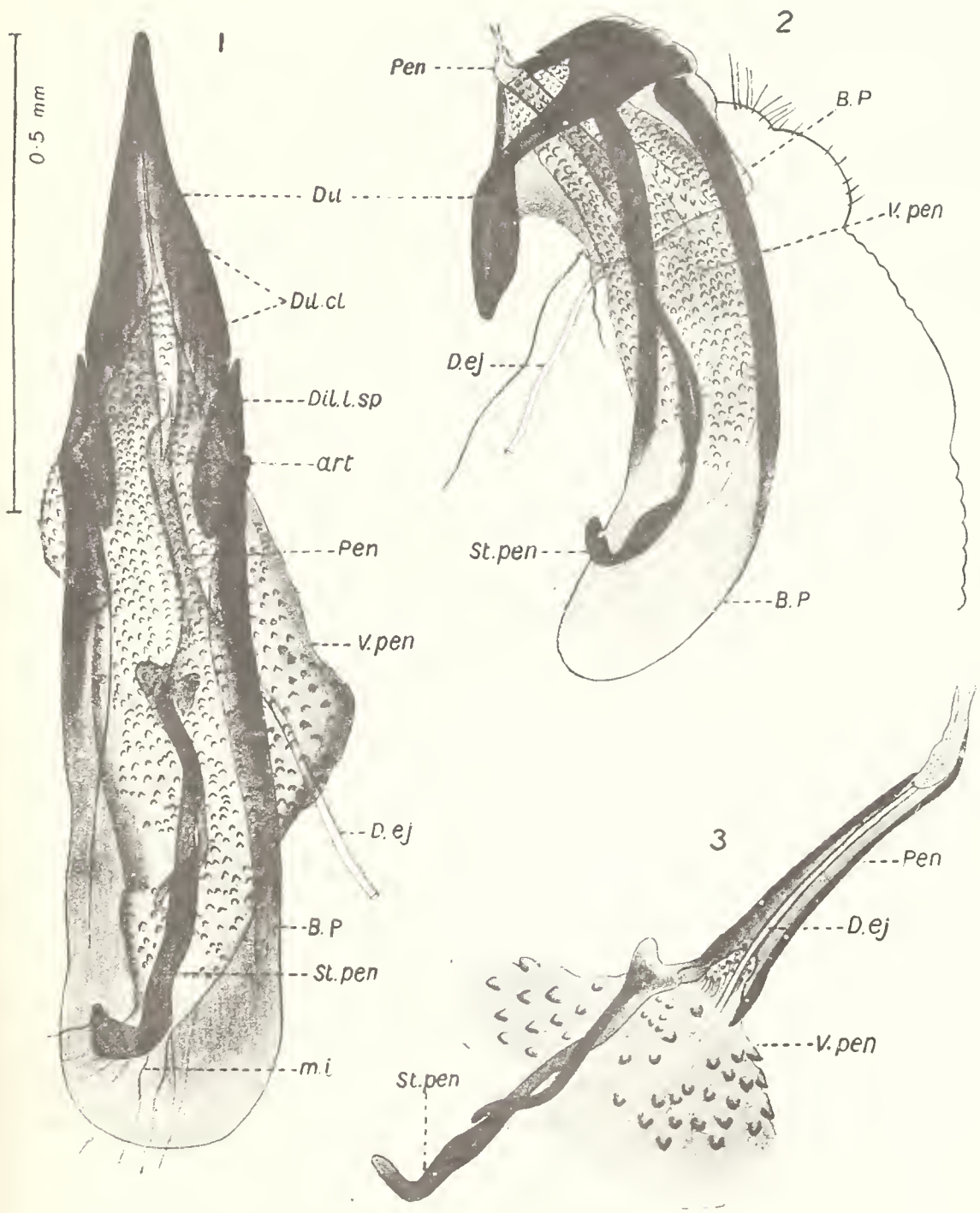
Penis: a term confined, in agreement with Mjöberg, to the chitinous tube which projects from the vesica (v. infra).

Vesica penis, or simply *vesica*, is a term applied in lieu of "preputial sack" (Mjöberg) and "sac interne" (Jeannel) both of which are misnomers though supposed to be descriptive.

Statumen penis is a term applied to the riblike chitinous thickening of the wall of the vesica penis (*Statumen* signifies a concrete object which causes a thing to stand, a support, stay, or prop).

These terms are purely descriptive. In this connection Sharp and Muir (1912, p. 485)[1] may appropriately be quoted, they rightly say in writing of the copulatory organs that it is "premature to establish permanent terms for the parts of the complex genitalia of insects till the various Orders have been more thoroughly examined and compared."

[1] See footnote, p. 298.



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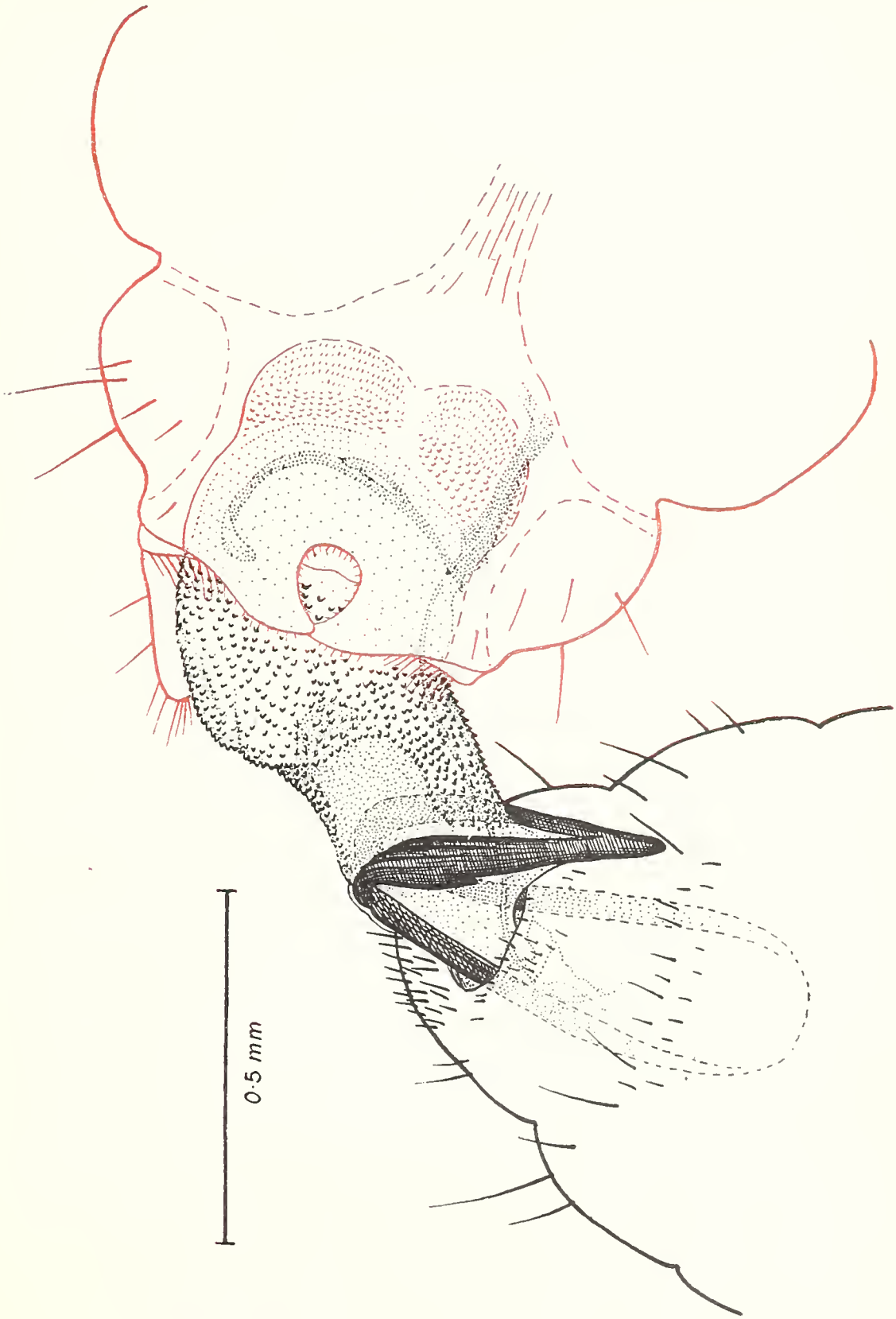

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Key to the Lettering Accompanying the Illustrations.

An. = anus.
art. = articulation.
B. P. = basal plate.
B. Pl. cl. = basal plate cleft.
D. ej. = ductus ejaculatorius.
Dil. = dilator (the parameres).
Dil. cl. = cleft in the dilator.
Dil. l. sp. = lateral spur on dilator.
Gl. acc. = accessory gland.
Gon. = gonopod.
int. = intestine.
ligt. = chitinous ligament fixing vagina.
M. d. a. = longitudinal dorsal abdominal muscles.
m. i. = muscular insertions in chitin.
M. constr. ut. = muscles, constrictors of uterus (transverse and oblique).
M. flex. dil. = muscles, flexors of the dilator.
M. pro. pl. = muscles, protractors of basal plate.
M. retr. d. v. = muscles, dorsal retractors of vaginal wall.
M. retr. pl. = muscles, retractors of basal plate.
M. retr. ut. = muscles, retractors of uterus.
M. retr. v. p. = muscles, retractors of vesica penis et al.
M. retr. v. v. = ventral retractors of vaginal wall.
M. v. a. = muscles, longitudinal ventral abdominal muscles.
ov. = ovaries.
p. g. f. = post-genital fold.
p. l. = posterior lobes of ninth segment (♀).
p. pl. = pregenital plate, facing pregenital fold.
Pen. = penis.
r. p. = rugose portion of vesica penis.
rect. = rectum.
segt. = segments of abdomen, numbered sixth to ninth.
sh. = collar-like sheath beneath protruded dilator.
St. pen. = statumen penis.
Stig. = spiracle.
trach. = trachea.
tr. f. = transverse folds of abdominal wall (♂).
Ut. = uterus.
V. pen. = vesica penis.
V. pen. f. = folds of inverted vesica penis.
V. pen. st. = stem of vesica penis.
v. p. = ventral plate over vagina.
vag. = vaginal orifice.
vag. fl. = vaginal flap.
vag.-ut. = vagina ends and uterus begins.
× = break in section, see legend to Text-fig. 3.

EXPLANATION OF PLATES III AND IV.

PLATE III.

Copulatory apparatus of *Pediculus humanus* ♂.

Fig. 1. Apparatus in its retracted state, treated with caustic potash, isolated, and viewed dorsally. The vesica is inverted and most of it lies in the hollow of the basal plate. It contains the penis which points into the cleft of the dilator. The ductus ejaculatorius issues on one side.

Fig. 2. Apparatus in situ, showing the first stage of extrusion of the vesica penis, the point of the penis appearing through the cleft in the dilator. Caustic potash preparation.

Fig. 3. Detail of the fully everted vesica penis, showing the penis with the therein contained ductus ejaculatorius, the statumen penis and a portion of the vesica with its strongly chitinized teeth. More highly magnified than the preceding figures to which the accompanying scale applies.

PLATE IV.

The relations of the ♂ and ♀ apparatus when in copula.

Drawing from mounted specimen of a pair killed in copula; the male parts printed in black, the female parts in red. For nomenclature of parts see preceding figures and text. Specimen treated with caustic potash.

Note all of these figures were drawn by me with the aid of a camera lucida. This remark likewise applies to the text-figures, excepting Nos. 9, 10 and 11.

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